THE IMPORTANCE OF URINE BIOCHEMISTRY AND RENAL HISTOPATHOLOGY IN THE AETIOLOGY AND MANAGEMENT OF UPPER URINARY TRACT STONES

John Alexander INGLIS

DOCTOR OF MEDICINE

UNIVERSITY OF EDINBURGH

1990

CONTENTS

" When the patient dies the kidneys may go to the pathologist, but while he lives the urine is ours. It can provide us, day by day, month by month, year by year, with a serial story of the major events going on within the kidney.

The examination of the urine is the most essential part of the physical examination of any patient....."

Thomas Addis (1881-1949)

ABSTRACT

Preface:

Urolithiasis is a condition known to Man for centuries, which once established, frequently pursues a recurring course. This might suggest a chronic underlying abnormality yet often no definite predisposing cause is found. Abnormalities in the urine, serum or renal histology of stone-forming patients are claimed by some to be aetiologically important while others continue to dispute this.

This thesis comprises a review of previous work, an account of a prospective study carried out by the author on a group of stone formers to address this problem and a discussion of the results obtained.

Synopsis:

Prior to the advent of an extra corporeal shock wave lithotripsy service (ESWL) in Scotland in 1986, most kidney stones were treated by percutaneous nephrolithotomy (PCN). This procedure provided an ideal opportunity to biopsy the kidney allowing renal histology and the degree of microscopic renal calcification to be assessed and compared with control material. The biopsy findings of individual patients were considered in relation to subsequent serum and urinary biochemical data. As the relative importance of various urinary risk factors pertaining to stone formation remains controversial, assessment of 24hr urine excretion of Calcium, Urate, Oxalate, Citrate and Creatinine, serum concentrations of Urea, Creatinine, Calcium and Urate and blood gas analyses were also undertaken in this group of stone formers. A group of non - stoneforming adult out-patients acted as controls.

Statistical analysis of all data was performed comparing the study group results both with our own controls as well as with other series.

This has allowed us to form conclusions about the process of stone formation and to develop a plan for the rational investigation and management of patients presenting with urinary tract calculi in this area.

SURVEY OF PREVIOUS WORK

Urinary tract calculi have been a cause of pain and morbidity at as far back as Egyptian times, the pattern and cause of stone formation being a subject for speculation and investigation for as long as records have been kept. Hippocrates noted that patients suffering from bladder or kidney stones often had "sand" in their urine and postulated that this resulted from the presence of Lime in the drinking water. Historical survey of the theories and results of later researchers (Butt 1956) reveals that by the 16th century, Jean Baptiste van Helmont had declared that stone formation resulted from excretion of an abnormal substance in the urine; the following century in 1684, Anton van Heyden had discovered something of the structural complexity of stones noting that removal of the crystalline component of a calculus left an underlying insoluble "framework". By the 1800's, chemical analysis of stones was well established and several different types of stone were recognised:- Calcium Carbonate, Phosphate of Lime, Oxalate of Lime, Ammonium Magnesium Phosphate, Uric Acid and Cystine. In addition, Meckel von Hemsbach had recorded that "stone formation depended on encrustation of an organic substance by precipitable material" and with the advent of diagnostic X-rays, the increased

incidence of stones in paraplegic and spinal injury patients and the association of stone formation with urinary stasis and infection was beginning to be recognised.

The anatomical location of stones within the urinary tract, shows a definite historical and socio-economic trend. Unlike the majority of present day stones which in 80% of cases present in the upper tract and progress distally in due course (provided the stone is small enough to pass), the urinary tract stone of former times was often a bladder stone de novo. Stones within the bladder have tended to occur most often in young boys in underdeveloped agricultural societies (Andersen 1973) and the removal of such calculi by the early Lithotomists is well described. As countries became more civilised and industrialised giving way to a more affluent society, the incidence of lower tract stones decreased and upper tract stones began to predominate.

The incidence of upper tract stones continues to increase in the Western world, Sallinen (1959) and Andersen (1966 and 1972) reporting an increased incidence in the first half of this century of 100% and 200% in Finland and Norway respectively; the prevalence has been previously assessed as 5-10% (Scott 1977, Tschope 1981, Sierkowski 1978), Smith observing in 1989 that by the age

of 70 years 5% of females and 12% of males can expect to have had renal colic.

The urinary calculus is a complex structure composed of one or more crystalline substances associated with a variable proportion of organic matrix material, the latter usually accounting for less than 10% of the dry stone weight.

The commonest age of presentation of stone disease is from the 3rd to the 5th decade and there is an overall male preponderance of 2:1, which increases to 3:1 when only Calcium containing stones are considered and to 5:1 when only the so called "idiopathic calcium stones" are studied. As Females in the above age band are mostly between the menarche and the menopause it may be that the difference in prevalence between the sexes is related to Oestrogen or secondarily to Citrate excretion. (see below). If primarily infective stones and those caused by confirmed Metabolic disorders such as Hyperparathyroidism or Cystinuria, are excluded from further discussion, the commonest stone type is that composed of Calcium Oxalate (with a small proportion of Calcium Phosphate) and this type comprises 60-80% of most series. Described as "idiopathic" because no single predisposing cause has been identified, (although many environmental factors contribute), this stone is almost exclusively the domain

of the young adult male, the condition pursuing an often recurring course throughout life, (Henneman 1958, Pak 1974, Rose 1976.)

It is generally believed that a Calcium Oxalate calculus develops within the kidney at a time when conditions are favourable and it may be pertinent to review theories of stone formation at this juncture. Firstly, the terminology needs to be defined :- when the solid phase of a stone salt is in equilibrium with the liquid phase, that concentration is described as the "saturation concentration". A less concentrated solution is said to be "undersaturated" whilst a more concentrated solution is described as "supersaturated". The "oversaturation concentration" is the maximum concentration which can be achieved without the occurrence of spontaneous precipitation, the product of the concentration of the relevant ions being called the "Formation Product". The range of concentrations intermediate between "saturated" and "supersaturated" is the "metastable region".

According to Pak (1969) and Robertson (1972), the urine needs to be supersaturated with respect to the specific ions comprising the stone before stone formation can occur.

This process is said to take place in 3 distinct phases (Robertson 1972).

i) Crystal Nucleation

ii) Crystal Growth

iii) Crystal Aggregation

The first of these refers to the formation of the smallest unit lattice of a crystal species and can be homogeneous or heterogeneous. In the case of the former, the contributing ions are "pure" while in the latter, the presence of a foreign body such as Collagen or a different crystal type can artificially lower the Formation Product allowing crystallization to occur. This latter process called Epitaxy was described by Lonsdale (1968) and Meyer et al (1975) although some other workers would question the validity of this theory. The observed association of Hyperuricosuria and Calcium Nephro-lithiasis was attributed to this phenomenon by Smith and Boyce in 1969 and also by Coe in 1974.

"Crystal Growth" is the process of enlargement of a nucleated crystal and also requires supersaturation conditions to be met thereby providing a setting for promoters or inhibitors to exert their effects.

"Crystal Aggregation" refers to the combination of a number of crystals to form the crystalline component of a calculus.

The rate of crystal growth in vitro has been shown to be proportional to the degree of supersaturation of a solution. In vivo, examination of freshly voided urine showed that crystalluria was more common in stoneformers than controls and that crystals passed by stoneformers were larger and more aggregated (Robertson 1969). Smith (1976) also noticed the passage of large aggregated crystals in 90% of stoneformers observing similar findings in only 20% of controls. The finding by Robertson (1972) of excess numbers of Calcium Oxalate crystals even in the calyceal urine of stoneformers suggests a proximal renal site for the initial event. It is assumed that there is some mechanism whereby an enlarged crystal becomes trapped during transit along the urinary tract allowing it to act as a nidus for further stone deposition, rather than being washed out in the urine before this can occur, but the mechanism for this remains unclear. In practice the two most consistently reported factors affecting stone formation are :-

i) The extent of urine saturation with a particular stone salt
(Robertson 1968/71, Finlayson 1969, Pak 1969)

ii) The concentration of protective Inhibitors and Promoters.

Thomas (1959) carried out in vitro experiments on the relative mineralizing properties of urine from stoneformers and controls; Fleisch (1962) isolated Pyrophosphate as an inhibitor of Calcification in urine and Meyer (1975) found evidence of a natural crystal growth inhibitor in Calcium Oxalate stoneformers' urine. In 1977, Ito and Coe identified an acid peptide / poly-ribonucleotide which they claimed was a potent inhibitor of crystal growth.

It has been frequently observed that stoneformers of a particular salt type have high urinary concentrations of one or more ions determining precipitability. Cystine stone formers excrete large amounts of Cystine in the urine (Harris,1953), (Dent,1955), (Crawhall,1969). Uric acid stoneformers have either a low urinary pH or excrete great quantities of insoluble Uric Acid (Metcalf- Gibson, 1965). Patients with infective (Struvite) stones which contain Ammonium, are frequently found to harbour bacteria which can split Urea in urine with the formation of Ammonia and a high urine pH. (Stamey 1972),(Griffith 1976).

With Idiopathic Calcium stoneformers, however, the picture is less clear-cut. According to some, firstly Flocks in 1939/40, followed by Albright and Henneman in 1953, with others since, (Hodgkinson and Pyrah 1958,

Bulusu 1970, Hodgkinson 1978), most recently including Nikkila in 1989, this group of stoneformers excrete greater amounts of Calcium and/or Oxalate in the urine compared with Controls. Lemann (1989) stated that 50% of stoneformers had urine Calcium greater than two standard deviations above the Mean control value. Others disagree, Robertson (1977) noting a considerable overlap between stoneformer and Control urinary Calcium levels suggesting that in addition to absolute urinary Calcium or Oxalate levels, other factors were involved as well. Welshman and McGeown (1975), Tiselius (1978) and Ryall (1983), however, could not detect a difference between stoneformers and controls.

The many epidemiological studies carried out world wide have established what environmental features predispose to stone formation. A low urine volume will tend to promote high super - saturation levels of Calcium Oxalate and has been shown to occur where input is low or in hot seasonal or climatic conditions where output falls as a result of skin losses. This would explain the seasonal variation in stone incidence noted in Australia and USA. Frank (1959) and Pierce (1945) observed an increased incidence of Urolithiasis amongst Israeli and American troops respectively when stationed in desert conditions. Parry (1975) however showed that as well as

causing low urine volume, transfer to a hot, sunny climate was also followed by significantly increased urinary Calcium excretion. Vitamin D3 is synthesised in skin exposed to sunlight, and is known to facilitate absorption of Calcium (and, indirectly, Oxalate) from the gut lumen. Hot climatic conditions can therefore promote stone formation in two ways. The importance of adequate hydration was stressed by Bek-Jensen in 1989 reporting that 25% of a series of Scandinavian stoneformers had 24hr urine volumes of less than 1000 ml.

It has also been shown that increasing affluence, social status and some occupations are all positively (but not necessarily independently) correlated with stone formation (Robertson 1981). All the above factors may simply reflect a more refined diet with a higher content of animal protein and dairy produce; Robertson has shown that such a diet leads to increased urinary levels of Calcium, Oxalate and Urate and feels that this factor alone could account for the observed increased prevalence of Urolithiasis in this section of the population. Other workers, however, have been unable to confirm this association between dietary animal products and Urolithiasis. (Griffith 1981 and Power 1984)

It has been suggested in the past that as the prevalence of Urolithiasis in Negro populations compared

with Caucasian races is very low, a racial or ethnic factor must exist. This has been refuted by Quinland (1945) who showed that the incidence of stone disease in Blacks became similar to Whites when they assumed a Western-style diet.

In summary therefore the major risk factors for idiopathic stone formation which can be identified epidemiologically, include being an adult male, leading an affluent life style, with a diet rich in animal protein and dairy produce and living in a relatively hot sunny climate where either as a result of ambient temperature or simply from consumption of too small a volume of liquid, the urine output is less than one litre per day.(Blacklock 1969)

Discussion of physio-pathological risk factors relating to calculus formation requires a careful appraisal of the source of origin, the renal handling and excretion of the various substances thought to be relevant. With idiopathic Calcium stones the metabolism of Calcium and Oxalate clearly merits close examination, however, the role of other suspected promoters or inhibitors such as Urate, Magnesium, Citrate or Mucoproteins must also be assessed.

Finally the histopathology of the normal and stone bearing kidney as evidenced from our own results as well

as by review of the literature will be examined to establish whether there exists any demonstrable abnormality amongst recurrent stone formers which might predispose to calculus formation.

CALCIUM

Calcium is derived mostly from dairy produce in the diet the net amount produced by endogenous bone breakdown in a healthy individual being minimal. Absorption from the lumen of the small gut is active to a large extent and dependant upon the influence of vitamin D3. Flux can proceed in either direction across the bowel, the net transfer being about 0.1 mmol/kg/24hr. Binding within the lumen of the bowel to Phosphate or fatty acids may restrict the availability of ionic Calcium for absorption. The amount of dietary Calcium available for absorption is increased by the presence of refined Carbohydrate in the form of Sucrose in the diet (Wasserman and Taylor 1969) as well as by the presence of animal protein, (Wasserman et al, 1956). Such an enriched diet was reported to cause hypercalciuria by Hodgkinson (1965) and Lindemann (1967). Lemann (1969) demonstrated an acute rise in urinary Calcium excretion after a dietary sugar bolus and Macleod and Blacklock (1979) showed a rise in Calcium absorption from the gut

following ingestion of Sucrose, although these were short term studies.

According to Andersen (1973) and Robertson (1978), the increasing dietary content of animal protein in the last 20 years is the major risk factor for calcium stone formation. The high protein/low fibre diet which characterizes the modern Western affluent lifestyle would seem to promote the absorption of dietary cations such as Calcium, perhaps because of the reduced amount of phytic acid which might otherwise form insoluble complexes with Calcium, preventing absorption, (James et al 1978).

Generally, 20% of dietary Calcium is absorbed by a combination of active transport and passive diffusion, the fraction absorbed by diffusion increasing with dietary Calcium content (Wilkinson 1976). At the kidney, 60% of plasma Calcium is filtered (250mmol/24hr) of which most is actively reabsorbed, 90% of reabsorption taking place in the proximal tubule linked to Sodium reabsorption. Factors preventing Sodium reabsorption such as Sodium load or loop diuretic e.g. Frusemide, reduce Calcium resorption at this site. A much smaller amount of Calcium resorption occurs in the distal nephron (distal convoluted tubule and collecting duct) where Sodium and Calcium movement are not linked. In fact Thiazide diuretics produce a Natriuresis without a Calciuresis

inducing a further distal tubular resorption of Calcium.(Stewart 1981)

Of great interest in relation to stone disease is the finding by some workers of abnormally high urinary excretion of Calcium in about 60% of stone formers, especially in Males. This so called idiopathic hypercalciuria is not associated with a raised serum Calcium and was first reported by Flocks (1940) and later by Hennemann (1958) who also observed an exaggerated calciuresis in this group of stone formers when challenged by an increase in dietary Calcium. It is perhaps surprising that whilst some workers are adamant that significant hypercalciuria is found in most idiopathic stoneformers (Hodgkinson,1978) yet others have found this not to be so. (Welshman and McGeown 1975, Tiselius 1978, Ryall 1983) It may be that this confusion arises from difficulty in defining normal values for Calcium excretion. It is known that the distribution of Calcium excretion in normal non stone forming individuals is non - Gaussian, there being significant numbers of apparently normal individuals with very high values. The generally accepted normal values (Hodgkinson 1958) for the U.K. are <7.5 mmols/24hr (adult males) and <6.25 mmols/24hr (adult females). Assuming that idiopathic hypercalciuria is a reality, there has been considerable

argument especially in North America as to whether this excess Calcium derives from over-absorption from the gut,"absorptive" as described by Pak in 1987 and Lemann in 1989 or because of a low renal threshold, "renal leak", a view held by Muldowney (1980). Current opinion favours increased absorption of Calcium from the gut as being the primary event in most cases. In addition idiopathic stoneformers are said to show an exaggerated Calciuresis in response to a dietary Calcium load, (Pak 1974/75). So called "renal hypercalciuria" requires an unspecified renal tubulopathy causing impaired resorption of Calcium, secondary hyperparathyroidism and as a result secondary hyperabsorption of Calcium from the gut. In fact these artificial divisions are probably oversimplified since if there was an obligatory renal leak, treatment in the past of such individuals with a Calcium gut binder e.g. Sodium Cellulose Phosphate (Dent 1964) would have caused severe demineralisation of the skeleton to maintain Calcium balance. Conversely if obligatory hyperabsorption from the gut was the causative factor in a given individual, treatment on an empirical basis with a Thiazide diuretic which reduces hypercalciuria by promoting Calcium resorption in the distal nephron,(Yendt 1970) would as a result have caused dangerous hypercalcaemia. As neither of these outcomes

has been reported the matter remains undecided. A practical policy in the management of Calcium stone disease with manifest hypercalciuria, (having excluded a primary hypercalcaemic cause) would be to increase fluid consumption until a urine output of 3 litres/24hr is achieved. In view of the association between dietary Calcium and hypercalciuria, animal protein and dairy produce should be restricted and in addition Oxalate containing foods should also be cut down as a result of the overabsorption which follows Calcium restriction (see below). If recurrent stone episodes continue despite these measures then treatment using Thiazides or Sodium Cellulose Phosphate would be indicated, (Pak 1974), (Hallson and Rose 1976), (Backman 1980). There has been some discussion in the literature recently regarding the possible attenuation of the hypocalciuric effect of Thiazides when used on a long term basis. In theory Thiazide should only be effective in renal hypercalciuria. Preminger in 1987 showed that the treatment of absorptive hypercalciuria with Thiazides was transient, attenuation of the initial improvement occurring over the subsequent two years. Conversely, similar treatment of renal hypercalciuria patients showed that the improvement was maintained indefinitely.

OXALATE

Oxalate is a substance which while found widely in the plant kingdom where it is useful in the formation of a supporting exoskeleton, is of little or no benefit to animals. It cannot be metabolised in Man thus any Oxalate which is absorbed from the diet or synthesised as a product of metabolism can only be eliminated from the body by excretion in the urine, where in view of the extreme insolubility of its Calcium salt, Calcium Oxalate, crystalluria and stone formation are not uncommon. Whether or not this occurs depends on the amount being excreted, the urinary volume and the relative concentrations of inhibitors of crystalluria which are present.

While the Oxalate content of some foods is known to be raised, e.g. Tea, Nuts and certain green vegetables, a recent paper by Brinkley, Gregory and Pak (1990) stresses that in relation to Nephrolithiasis, bioavailability as well as content must be considered. Their study of the content and bioavailability of Oxalate in tea (with or without milk), turnip greens, okra, peanuts and almonds concludes that overall, only nuts present a significant risk of raising Oxalate excretion in the urine. Robertson in 1979 showed a relationship between urinary Oxalate and dietary protein content, however, this was not

substantiated in a recent report by Marangella in 1989. Normally urinary Oxalate excretion is less than 0.45 mmol/24hr. Hyperoxaluria exists when larger amounts are present in the urine daily, either as a result of an inborn error of metabolism (Primary Hyperoxaluria) or as a secondary phenomenon such as unusual dietary intake or small bowel disease or surgery leading to increased absorption from the gut. The commonest form of Primary Hyperoxaluria arises from the absence in the liver of the peroxisomal enzyme, Alanine - glyoxylate aminotransferase resulting in the presence in the urine of large amounts of Oxalate (0.8-4.0 mmol/24hr) as well as the metabolite Glycollate (>0.33 mmol/L). In secondary Hyperoxaluric states the urine excretion seldom exceeds 1.0 - 1.5 mmol/24hr. and in these circumstances, Glycollate is not raised.

Some workers believe that the way in which Oxalate is handled is at least as important as that of Calcium excretion with respect to idiopathic stone formation. Robertson (1968) observed that while stoneformers' urine was generally more concentrated with Calcium than Controls the increase was not sufficient to cause spontaneous crystallisation. Moderate increases in urinary Calcium concentration caused no increase in Calcium Oxalate crystalluria. Similar increase in urinary

Oxalate levels, however, caused significant increase in Calcium Oxalate crystalluria, suggesting that Oxalate might be more important than Calcium in this respect. The sources of origin of urinary Oxalate are less well understood than for Calcium. It is known that only 5-10% of Oxalate present in the gut is normally absorbed, possibly because it is complexed with Calcium in the lumen and passes out unchanged. Furthermore the amount of intraluminal Calcium determines the percentage of Oxalate absorbed there being an inverse relationship between ingested Calcium and Oxalate absorption. (Williams and Wandzilak 1989). In conditions of small bowel pathology or following small bowel resection this figure may rise to 60%. This enteric hyperoxaluria is thought to be due to Calcium being bound to unabsorbed long chain fatty acids and as a result Oxalate remains un-complexed and thus available for absorption. In a similar way enhanced uptake of Oxalate occurs following dietary restriction of Calcium. Another suggested cause of enteric hyperoxaluria is the excessive absorption of Oxalate from the colon as a direct result of mucosal change caused by the presence of unresorbed bile acids, (Dobbin 1976). In addition to gut absorption, urinary Oxalate is partly derived from endogenous metabolism although the absolute amount forming via glycine or ascorbic acid pathways is

controversial (Hodgkinson 1977). Rose (1988) concludes that endogenous production is only significant on a low Oxalate diet and points out the errors that may have previously arisen by assuming that Oxalate identified in the urine had derived from Ascorbate metabolism in vivo when in fact Ascorbate in urine converts spontaneously in vitro at alkaline pH (Rose 1985). It follows therefore that in the past, spuriously high Oxalate level may have been recorded from 24hour urine collections not acidified ab initio.

Oxalate reaches the urine both by filtration and tubular secretion and given favourable conditions of osmolality and pH forms typical octahedral crystals (envelope crystals) characteristically of 2-10um in size although large crystals >100um across have been described. It is said by some that the smaller crystals are common in normal individuals and that the larger sized crystals are found only in stone formers, perhaps as a result of a deficiency of inhibitors (Robertson et al 1969). These crystals remain discrete by virtue of mutually repulsive electrostatic potentials and it may be that inhibitors exert their effect by increasing this potential. It has been noted that microscopy of centrifuged, fresh whole urine, after evaporation, reveals clusters of Calcium Oxalate crystals but that

this appearance is not seen if the urine is previously subjected to ultrafiltration (pore size 12000 daltons) (Hallson and Rose 1979). When uromucoid is returned to the solution the cluster phenomenon is seen once more and this is thought to be due to a high molecular weight mucoprotein -the Tamm-Horsfall mucoprotein, triggering the nucleation of crystals of Calcium Oxalate and Calcium Phosphate on strands of insoluble polymerised uromucoid. Experimental evidence suggests that the degree of crystallization is also pH dependent, increasing with pH (although this is partly in association with Calcium Phosphate crystals). Further experimental evidence shows that while increasing crystallization can be achieved by increasing the concentration of either Calcium or Oxalate in a study solution, Calcium levels have to be raised to a concentration corresponding to severe hypercalciuria in vivo to cause this effect. A much greater increase in crystallisation is achieved by raising Oxalate levels to a lesser extent, corresponding to a urinary Oxalate just above the normal range, (Robertson 1968). This would suggest that while both Calcium and Oxalate levels are important, that perhaps Calcium Oxalate crystalluria is more critically sensitive to changes in Oxalate concentration. In view of earlier difficulties in the assay of Oxalate the reported prevalence of significant

hyperoxaluria in stone formers varies from 2 - 3 % to as much as 40 - 50 %, (Bailey 1974, Robertson 1980, Wallace 1981, Baggio 1983 and Pena 1987). Williams and Wandzilak (1989) concluded that a figure of 10 -20% was more likely which would agree with the figure of 17% reported by Bek Jensen in 1989.

URATE

Urinary Uric Acid is derived from protein in the diet or from purines produced by endogenous breakdown. In either event purines are first dephosphorylated to form Hypoxanthine which is oxidised to Xanthine and then to Uric Acid, both oxidation reactions being controlled by Xanthine Oxidase. The Urate so produced is passed from the plasma to the kidney where it is secreted in the urine.

It is well described that patients with abnormal purine metabolism excrete increased amounts of uric acid in the urine, often resulting in the formation of non - calcified urate stones. Such patients characteristically have acidic urine favouring the formation of relatively insoluble uric acid.

Sodium Urate ----------> Uric Acid

High pH pKa= 5.75 Low pH

The management of such stone formers is to encourage a high urine output and to increase urinary pH to <6.5 in order to promote formation of the more soluble ionised urate (pH values of >6.5 will cause crystallization of calcium phosphate upon pre-existing Urate calculi.) An additional treatment is the long term ingestion of Allopurinol a drug which specifically inhibits Xanthine Oxidase thus blocking uric acid production.

Much more controversial is the contention that urinary Urate levels are raised in idiopathic Calcium stone disease, and play an active part in mediating Calcium Oxalate stone formation. Coe (1978) felt that there was an association, as did Robertson in 1978, reporting significant hyperuricosuria in Calcium Oxalate stone formers. Hodgkinson (1976), Strauss et al (1982) and Fellstrom (1982) however drew opposing conclusions. Hallson and Rose (1982) found no effect of Urate on Calcium Oxalate crystallisation in whole urine while Pak (1979) and Ryall (1986) report otherwise. The confusion may partly be explained by the difficulty in establishing a normal reference range. Not only do "normal" values vary geographically travelling West to East in Europe but normal values in a defined population and region seem to have increased over the last few decades. This trend may simply reflect increasing affluence with Man's

predilection for animal protein and underlines the perils awaiting the unwary epidemiologist who might be tempted to compare geographically distinct study populations or use historical controls.

Most Western European laboratories quote an upper limit of normal Urate excretion as 4.7 mmol/24hr ,however recent series suggest that levels up to 7 mmol/24hr are not unusual.

Fleisch (1978) proposed that Calcium Oxalate crystalluria might be promoted by the process of Epitaxy whereby different substances whose crystal lattice structures are of similar dimensions can mutually enhance the crystallisation of the other. Pak (1979), on the other hand, suggested that the inhibitor capacity of Glycosamino-glycans (GAGs) in urine was reduced by adsorption by Urate so enhancing Calcium Oxalate crystallisation.

Despite the controversy surrounding the mechanism involved, there is some clinical evidence to support a role for Urate in idiopathic stone disease. In recent controlled trials a reduction either in Oxalate excretion or in episodes of stone recurrence is claimed in response to treatment of idiopathic stone formers with Allopurinol (Tomlinson 1985), (Scott 1978 and 1989). This is theoretically possible since Xanthine Oxidase is one of

the endogenous enzymes capable of catalysing the oxidation of Glyoxylate to Oxalate ; an inhibitor of Xanthine Oxidase such as Allopurinol could therefore reduce Oxalate synthesis and ultimately, urine Oxalate levels. A recent publication by Urivetsky (1990) while concurring with the observations of Scott and Tomlinson et al in demonstrating a fall in both urinary Urate and Oxalate after treatment with Allopurinol, showed in a follow-up study that the difference could have resulted from change in diet alone, and was not dependant on Allopurinol. Raised levels of Oxalate excretion were noted in individuals consuming a high protein diet and vice versa whether on or off Allopurinol. While it is accepted that Allopurinol will reduce Urate excretion in hyperurocosuric Calcium Oxalate stone formers it remains uncertain whether this reduces the rate of stone recurrence. Finlayson (1985) and Hofbauer and Zechner (1988) felt there was no evidence to support such a claim. A recent report by Ettinger (1989) agreed that the only examples of improvement in recurrence rates were found where Allopurinol had been given selectively to normo - Calciuric patients, and that no case had been made for the use of Allopurinol in the treatment of stone formers exhibiting both hypercalciuria and hyperuricosuria. This is particularly relevant in view of

the reported increase in allergic reactions when Thiazide and Allopurinol are used in combination.

INHIBITORS

As well as researching the positive factors relating to crystalluria and stone formation considerable effort has been exerted in order to establish the role of negative factors or inhibitors. If such substances are present and active at the physiological concentrations found in the urine of normal individuals, their deficiency or absence, observed in some stone formers may be significant. Many urinary constituents have been suggested as inhibitors i.e. protective colloids, (Butt 1952), small ions such as Citrate, (Howard and Becker 1976), Magnesium, (Muckai and Howard 1963), Pyrophosphate, (Fleisch and Bizaz 1964), anionic macromolecules, (Robertson 1973/1976) such as GAGs, RNA, acidic glycoproteins and non-polymerised Tamm-Horsfall mucoprotein.

Inhibitors are thought to act by adsorbing to the surface of crystals slowing down the kinetics of growth and agglomeration (Scurr and Robertson 1986). Citrate as well as acting in this way regarding Calcium Oxalate and Calcium Phosphate crystals also reduces the risk of stone formation by forming soluble complexes with Calcium(Ca++)

thus reducing the ionic concentration. Magnesium and Sodium form similar complexes with Oxalate (Smith 1989). Magnesium and Citrate therefore exhibit the dual effect of inhibition and complexation (Nancollas 1976). There have been many claims and counter claims based on investigations and treatments arising from them.

MAGNESIUM

An early possible inhibitor to be considered was Magnesium. Hammarsten (1929) noted that in simple solution it formed soluble complexes with Oxalate and reduced Calcium Oxalate crystalluria and these findings were supported some years later by Fleisch (1978) and by Hallson et al (1982). On the other hand, this view was not supported by Sutor's experimental results in 1970. Good results of treatment with Magnesium compounds have been claimed by Melnick (1975) and Johannson (1980) and most recently a mixture of Magnesium and Tartrate has been advocated by Hallson and Rose (1988) which resulted in an increased level of urinary Citrate and Magnesium and a reduction in urinary Calcium.

MUCOPOLYSACCHARIDES

Another group of substances found in the urine and proposed as having inhibitor properties are the Mucopolysaccharides. Robertson et al reported in 1973 that such a substance inhibited the agglomeration of Calcium Oxalate Monohydrate crystals in vitro, however, subsequent reports have been unable to agree. Bowyer (1979) nominated Chondroitin Sulphate as a possible example and it has been claimed by some that the stone promoting ability of Uric Acid relies on blocking the inhibitor action of this group of substances. Fleisch in 1978 distinguished between "precipitation" and"aggregation" stating that while for both Calcium Oxalate and Calcium Phosphate stones the important inhibitors of precipitation were Citrate and Pyrophosphate, GAG's were important inhibitors of aggregation. Sutor in 1979 in a set of "whole" v "synthetic" urine experiments while agreeing with the action of Citrate and Pyrophosphate could find no evidence either of an occult, unidentified inhibitor nor of any such effect with GAGs. At about the same time Pak reported the results of his studies on two macromolecules, namely Heparin and Chondroitin Sulphate, the latter having been suggested the previous year as a likely Inhibitor, (Bowyer 1979). According to Pak, the

marked inhibitor action of Heparin could be demonstrated in test solutions, as evidenced by the rise in the Formation Product of Calcium Oxalate which occurred when Heparin was added to the solution, at a concentration of 0.05mg/litre. In addition, this effect could be blocked by prior incubation of the mucopolysaccharide with Monosodium Urate although this latter effect required a Urate:Heparin ratio of 2000:1 which would be unlikely in physiological conditions.

In 1981 Koide reported the results of his experiments to assess the degree of inhibition of aggregation found in the urine of stoneformers and controls. The urine was first filtered to remove such low molecular weight substances as Citrate and Pyrophosphate and so isolate the effect of urinary macromolecules (m.w.>10,000). He found a much greater inhibitory effect in the non stone formers' urine which could be abolished using a protease, suggesting that it was indeed a protein complex, however, urinary acid GAG's were thought not to be important inhibitors.

Ryall (1984) compared the inhibitory activity of urine from stoneformers and Controls, specifically looking at levels of GAGs, Urate and any mutual interaction. While identifying degrees of inhibitory activity, she could find no difference between

"unselected" Stoneformers and Controls, concurring with Koide (1979) and Sallis (1979) that macromolecules other than GAGs had a more important inhibitory role. In 1988, Hwang and Preminger et al also reporting a large series of quantitative urine analyses, could find no difference between stoneformers and controls. Nikkila in 1989 found that when recurrent stone formers were examined separately from first time stone formers then a significant reduction in GAG excretion compared with controls could be demonstrated, supporting the "selective" findings of Ryall.

The role of Macromolecules as Inhibitors thus remains contentious; no attempt has been made to establish further their relevance to Urolithiasis during the course of this study.

PYROPHOSPHATE

Pyrophosphate has been claimed by some (see above) to be an inhibitor of stone formation on the basis of in vitro experimental work, however others feel that sufficiently high concentration needed to be effective in this capacity could not be achieved under physiological conditions. Hallson and Rose (1983) concluded that Pyrophosphate had no role to play in modifying Calcium Oxalate or Phosphate crystal formation in whole urine. It

may be that any effect however small, is exerted by adsorption on to crystal surfaces preventing aggregation as suggested by Sutor in 1979.

CITRATE

Citrate is another normally occurring urinary constituent which is said to have a role as a natural inhibitor of stone formation and there are several ways in which this effect might be achieved. Its excretion reflects renal intracellular metabolism and acid-base status (Simpson 1983) and varies with the acid content of the diet (Gamble 1961). Renal acidification abnormalities are not uncommonly associated with nephrolithiasis and in such patients hypocitraturia is often a feature. (Backman 1961). Hastings showed in 1934 that Citrate formed soluble complexes with Calcium reducing the urinary saturation with Calcium salts and there is also evidence that it may inhibit the growth and/or aggregation of crystals of both Calcium Oxalate and Calcium Phosphate, (Meyer 1975, Felix 1977, Ryall 1985, Kok 1986.) Hallson and Rose noted that Citrate significantly reduced both Calcium Oxalate and Calcium Phosphate crystal formation in vitro using a rapid evaporation technique (1983).

There have been difficulties to overcome, however, both in technique and interpretation especially regarding

the early reports. The method of assaying Citrate using Bromacetone (Natelson 1948) proved to be somewhat unsatisfactory therefore early reports on Citrate levels are less reliable than later studies carried out after the introduction of a specific enzymatic method of estimating Citrate using Citrate Lyase. (Moellering 1966) In addition, it has been shown that certain bacteria can degrade Citrate, thus post renal destruction of Citrate can occur, either in vivo in the presence of an UTI or when completed urine collections become contaminated before analysis, both circumstances resulting in spuriously low Citrate levels. Conway et al (1949) concluded that urine infection accounted for all cases of hypocitraturia, however, Hodgkinson (1962) claimed that low urinary Citrate excretion was more likely to reflect impaired renal function. Certainly later studies have shown a relationship between renal failure and low urinary Citrate as a result of reduced filtered load. Buckalew in 1989 showed that in patients with Renal Tubular Acidosis (RTA), characterised by low urinary Citrate, there was a linear relationship between plasma Bicarbonate and Creatinine clearance (provided Creatinine clearance was less than 80 mls / minute.)

Shorr et al (1942) showed a marked difference in urinary Citrate levels between males and females as a

result of ovulatory oestrogen rises, pointing out that if groups of stoneformers were not stratified for sex before analysis then serious errors could arise. In addition Hosking (1985) reported a rise in Citrate excretion with increasing age in both sexes in normal individuals which was not seen in stone formers of either sex.

In 1976 Welshman and McGeown reported their results taking account of all the above pitfalls and concluded that while there was a significant difference in Citrate excretion between (especially young) male and female adult Controls, there was no significant difference between Male and Female stoneformers. When compared with controls, however, there was overall a degree of hypocitraturia in stone formers which reflected neither infection, poor renal function nor inadvertent sexual bias. In addition they observed a significant linear relationship between urinary Calcium and Citrate excretion in all groups examined, but particularly marked in young adult females (see Results of this study). In 1979 Schwille et al reported a significant reduction in Citrate excretion in a group of recurrent Calcium Oxalate stoneformers compared with Age and Sex matched Controls. Nicar (1983) also demonstrated significantly low Citrate levels in approx 50% of an unselected group of stoneformers of differing aetiologies, except in Calcium

Oxalate stone formers who were also hyperuricosuric (see results of this study). He also observed significant correlation between Citrate and Calcium excretion ($p<0.025$), surmising that divalent cations such as Calcium and Magnesium might enhance Citrate excretion by forming complexes with Citrate and thus preventing its reabsorption. In the same year Menon and Mahle (1983) reported significant hypocitraturia in 15% of a group of Calcium Oxalate stone formers of whom 13% had no other demonstrable abnormality. This was followed two years later when Pak (1985) reported the results of treating a group comprising only Calcium nephrolithiasis patients, with Potassium Citrate. Not only was urinary saturation with Calcium Oxalate reduced and the propensity for spontaneous nucleation of Calcium Oxalate diminished (as shown in vitro by experiments using the urine of treated patients) but the rate of stone episode recurrence was reduced in 80% of the treatment group. This latter aspect of this study is open to criticism as there was no non-treatment group and such apparent improvement may easily arise simply as a result of a placebo or "stone-clinic" effect.

In 1986, Kok reported a set of experiments using the urine of controls and stoneformers, the only detectable abnormality in the stoneformer group being

hypocitraturia. Using a seeded crystal growth system he was able to assess separately, degrees of both inhibition of crystallisation and aggregation. While both control and patient urines were equally good at inhibiting crystallisation, the stone-formers' urine was significantly poorer at preventing aggregation, suggesting that the Citrate was acting as an inhibitor of crystal aggregation in the control group.

Regardless of its place in the aetiology of idiopathic stone disease, the observation of low Citrate levels in conditions associated with renal acidification defects which themselves often accompany Nephrolithiasis, is well described (Fourman and Robinson 1953, Backman 1980, Buckalew 1989). The commonest example is the distal variant of type 1 Renal Tubular Acidosis although similar defects in acidification have been reported in association with Medullary Sponge Kidney (MSK) (Osther 1988). In type 1 RTA the major abnormality is the inability to create a H+ ion gradient between tubular cells and urine in the presence of acidaemia. If the lesion is complete the clinical picture is one of Hyperchloremic Acidosis, Hypercalciuria, High urinary pH, Hypokalemia and Hypocitraturia. The urinary pH cannot be reduced below 5.3 (Urine can be acidified to pH of 4.5 in health) and fixed bases, Na+, K+ and Ca++ are lost in the

urine (Dedmon 1962) (Morrissey 1963) (Buckalew 1989).

The incomplete distal form of the condition is the only significant variant which is relevant to Nephrolithiasis. There is no overt acidosis, the diagnosis relying on a challenge with an oral acid load in order to prove the defect in urinary acidification capacity. Incomplete distal RTA is infrequently reported in the UK, possibly as it is seldom sought, however, according to the literature it occurs in up to 20% of presentations with renal stones in North America and Scandinavia (Preminger 1985) (Backman 1980), although whether it is the underlying cause, or arises from secondary to renal pathology is less clear.

Buckalew (1989) classifies type 1 RTA as being Hereditary, Idiopathic or Secondary, stating that secondary forms are often associated with autoimmune disorders and occur more frequently in female subjects. This would be in keeping with the findings of Backman et al (1980) who in a study of 318 recurrent stone formers found impaired renal tubular function in 19 % of cases, most of whom had incomplete type 1 RTA. This impaired renal acidification occurred in 13 % of male stone formers and in 38 % of female stone formers.

While it would be facile in this complex condition to claim that all cases of Nephrolithiasis were due

solely to Hypocitraturia, it is noteworthy that Preminger and Pak (1985) have reported excellent results from treating RTA with the alkali Potassium Citrate orally, demonstrating an objective reduction in urinary Calcium excretion, an increase in urinary Citrate and a lowering of the relative urinary saturation product of Calcium Oxalate. The use of Sodium Citrate, while increasing Citrate excretion, did not bring about a corresponding reduction in urine Calcium concentration by virtue of the increased Sodium load. It is relevant at this point to mention a less desirable consequence of Thiazide treatment in the management of idiopathic hypercalciuria. The secondary hypokalemia results in hypocitraturia, thereby possibly reducing one stone risk factor at the expense of raising another. This complication can be avoided by the use of Potassium supplements in combination with Thiazides and clearly Potassium Citrate would be an appropriate choice.

In summary therefore it appears that Citrate complexes with Calcium and restricts Calcium Oxalate stone formation by in this way as well as by disturbing the process of crystallisation and/or aggregation, although it may be that in some series reported in the past that spuriously low levels of urinary Citrate in fact resulted from urine infection, poor renal function

or failure to take note of sex differences.

It is to be hoped that in the clinical study that forms the basis for this Thesis adequate notice has been taken of the errors and pitfalls which beset earlier workers and that this work will provide an accurate reflection of the relevance of citrate excretion in stoneformers from East Central Scotland.

RENAL CALCIFICATION

In the search for an underlying cause of renal calculi, attention has been focused not only on the composition of the urine but also on the renal substance itself, which has come under scrutiny to try to gain a clue as to the mechanism of stone production. To this end, radiological and both light and electron microscope studies have been carried out on normal as well as stone-formers' kidneys. Commencing with Virchow's report of calcified renal deposits which he described as 'Kalk Metastasen' in 1855, the initial site of formation and subsequent progress of microcalculi within the kidney has been a subject of intense debate. In 1936 Randall described the presence of macroscopic subepithelial plaques of calcification situated on the papillae of 20% of kidneys examined and this work was followed by the microscopic studies of Anderson and McDonald (1946) where

some degree of calcification was demonstrable in all patients examined (although this study of 168 patients was confined to the renal medulla). Haggitt and Pitcock (1971) conducted a pathological study of 100 kidneys removed at autopsy. This study was also confined to medullary examination and again no reference was made to a history, or not, of previous nephrolithiasis. Varying degrees of calcification, confined to the basement membranes and interstitia of collecting ducts, was noted in all cases, the Electron Microscope appearance being dense laminated spherules which were very rarely located within the Nephron. Malek and Boyce in 1973 published the results of their studies in which the findings in stoneformers and non-stoneformers were considered separately. In a group of 64 patients with nephrolithiasis of varying aetiology calcified deposits were noticed within the proximal and distal tubular cells as well as within the tubular lumina in 52/64 (81%) of stoneformers overall, however this corresponded to 100% of those patients with Calcium Oxalate lithiasis there being no deposits in those with a history of Infective (Struvite), Urate or Cystine stones. In this study there appeared to be a gradient of microcalcification increasing from the Cortex towards the Medulla which was maximal at the papillary tip. In the Control group, in

the 29 who had no history of stone disease, only 3 (10%) showed evidence of microcalcification. He postulated that the initial event was the formation of a microcalculus within the mitochondria of the renal tubular cells which subsequently disrupted the organelle to occupy the tubular cell. The tiny concretion resulting from aggregation of several of these microcalculi might then rupture into the tubular lumen of the parent nephron whence it could migrate towards the medulla, passing via the papillae into the urine or becoming occluded at the level of the collecting ducts or in a subepithelial distribution to form a Randall's plaque. This putative sequence of events received a measure of support from animal studies performed by Caulfield (1963) when renal calcification was artificially produced by intra peritoneal injections of either PTH or Calcium Gluconate in mice. The animals were subjected to varying frequencies and concentrations of the injections and were sacrificed at intervals when E/M studies of the kidneys were carried out. In this study the earliest appearance of calcium deposition was within the mitochondria of tubular cells, intraluminal calcification being seen later. It is worthy of note however that the earliest sacrifice was performed at 48 hrs after injection. In a similar study by Khan et al (1979) intraperitoneal

injections of Sodium Oxalate were given to rats in order to induce renal Calcium oxalate crystal formation. In this latter study crystal formation was shown to be primarily an intra-luminal phenomenon confined to the proximal tubules of the Cortex with subsequent progression distally towards the papilla. This finding was demonstrated in animals sacrificed only 15 minutes after injection; crystals were found in renal tubular cells and within the interstitium only in animals sacrificed at 7 days or longer after injection. That interstitial Calcium deposits can arise secondary to intraluminal Calculosis was previously asserted by Roberts (1976) and Epstein (1971).

In a personal study (Harrison and Inglis, 1988) renal biopsies were taken from stoneformers at the time of PCN and compared with Controls. In our preliminary study, Calcification was present in 72% of Stoneformers but only 23% of Controls. The calcified deposit was either a small amorphous body present within tubular lumina, tubular cells, or rarely within the interstitium, a much less common form being found in relation to the basement membrane of Bowman's capsule. Electron microscopy was carried out on 5 cases in which light microscopy had demonstrated calcified deposits. There were no Ultrastructural abnormalities but small electron dense bodies were demonstrated within tubular cell

basement membranes and mitochondria which were larger and more numerous than mitochondrial bodies observed in Controls. In the Control patients with no history of stone disease Calcium deposits were very much rarer and were mostly confined to the collecting tubules within the Medulla.

In conclusion therefore it would appear that there is a reasonable consensus amongst the published evidence over the last 50 years that while small deposits of Calcium can be detected microscopically in kidneys of non stoneforming individuals, most commonly in the region of the renal papillae, the incidence and extent of such calcification is increased dramatically in stoneformers, particularly of Calcium Oxalate type. A possible sequence of events comprising a cellular or biochemical abnormality in the urine occurring within the lumen of a cortical tubule or tubular cell, allowing formation of a tiny concretion. This tiny calculus which either primarily or secondarily reaches the lumen, subsequently progresses distally to the papillae to be voided as microcalculi in the urine. Their course however may become impeded at various points along this route and if such occurs they may form a nidus for further aggregations of small concretions with subsequent development of a renal calculus.

PATIENTS, MATERIALS and METHODS

In Edinburgh prior to the establishment of an extra-corporeal Lithotripsy service, the majority of Renal Calculi requiring active intervention were treated by Percutaneous Nephrolithotomy (PCN). The operation of PCN is carried out under G.A. usually as a single stage procedure with antibiotic cover (Inglis 1988). The renal collecting system is first imaged fluoroscopically by injection of contrast via an ureteric catheter passed at the time of preliminary cystoscopy. After turning the patient prone the opacified system is punctured through the most favourable calyx and after passing a guide wire into the collecting system, graduated dilators are passed serially until a track large enough to accommodate an Amplatz tube is created. The nephroscope is passed along this latter tube and using fibre optic illumination and saline irrigation the stone can be visualised and either removed intact or piecemeal, following preliminary fragmentation using an Ultrasound or Electro-hydraulic probe.

At the end of the procedure and before insertion of a nephrostomy tube for drainage, endoscopic biopsies of the renal parenchyma were taken using standard cup-biopsy forceps at a site distant from the location of the stone and from tissue which was of normal appearance.

The nephrostomy tube was removed when the urine drainage was clear and the patient discharged home 24 hours later. Further assessment of urinary parameters was undertaken as ambulatory outpatients.

Renal Biopsies

Renal biopsies were performed in 69 stoneformers overall (48 Male and 21 Female) however a number of patients were lost to follow up or failed to complete subsequent urine collections, therefore comparative Data on Biopsies and Urine chemistry is available on 47 patients. The biopsies were examined using light microscopy to seek evidence of renal parenchymal micro-calcification, any associated pathology and their relevance to urine biochemical abnormalities and Nephrolithiasis. In addition some sections showing evidence of Calcification on light microscopy were also examined by Electron Microscopy. The histo pathological assessment was conducted throughout by a single pathologist (D. H.)

The biopsies were initially fixed in 10% buffered formalin and embedded in paraffin wax. Calcium was demonstrated using two techniques; the first, using von Kossa silver, substitutes a dark brown silver deposit for carbonate or phosphate, and while not specific for

Calcium is regarded as valid (Pearse 1972). The second complementary method uses Alizarin Red which at a pH of 4.2 dissolves Calcium deposits to form red calcium lakes (Pearse 1972). Control renal tissue was also examined for comparison of the relative amount and site of calcification compared with stoneformers. This material was obtained from 30 renal biopsies (either open-wedge or needle) carried out on patients suffering from any of a number of mixed renal pathologies excluding nephrolithiasis i.e. ATN, Hypertension and Glomerulonephropathy. In addition two kidneys obtained at necropsy and one removed surgically for tumour were assessed by multiple biopsies to confirm that a single cup biopsy was representative of the kidney as a whole.

Urine and Blood Analyses in Stoneformers

The patients included in the above study were 87 consecutive adult idiopathic stone formers attending RIE for PCN. The group who were all resident in East Central Scotland comprised 58 Males and 29 Females. They all had radiographic evidence of Calcium nephrolithiasis, were unselected regarding stone size and were otherwise in good health with normal renal function. They were, however, excluded from the study if there was any past or present history of urinary infection. Any patient found

at preoperative screen to be Hypercalcaemic was referred for appropriate investigation of the cause and excluded from this study of idiopathic stone formation.

This group of patients had had no specific dietary advice regarding stone prophylaxis and were on no medication thought to affect stone precursor metabolism i.e. Thiazides, Cellulose Phosphate etc.

At the time of Out-Patient review a series of additional biochemical investigations was arranged and supervised personally. These included estimation of serum Calcium, Urate and Urea and/or Creatinine (to confirm normal renal function) as well as Arterial blood gas analysis. 24 hour urine collections were completed in containers to which 50ml of 5M HCl had been added to prevent any bacterial degradation of Citrate in vitro and also prevent any conversion of Ascorbate to Oxalate. All collections were returned to me so that the volume could be measured and recorded. Two 20 ml aliquots were deep frozen for subsequent Oxalate and Citrate assay, the estimation of Calcium, Urate and Creatinine being performed locally in the Biochemistry Dept.of the Royal Infirmary of Edinburgh.

Under the supervision of Professor L.G. Whitby, serum Calcium and Urate as well as urinary Urate was analysed using a Technicon Simultaneous Multiple Analyser

with Computer II (SMAC II) system and urinary Calcium measured on a Technicon RA - 1000 Random Access Analyser (Technicon Instruments Co., Basingstoke, Hants.)

Arterial blood gas specimens were drawn solely by myself using disposable pre-heparinised vacuum syringes from radial artery punctures after infiltration with 2% Lignocaine. The samples were delivered to the laboratory personally, on ice, within 30 minutes of sampling and analysed using an IL 1312 automatic blood gas analyser (Instrumentation Laboratories, Warrington, Lancashire).

It was not possible for this laboratory to perform assays for urinary Citrate or Oxalate. Thanks to the generous cooperation of Mr Ian Gibb, Principal Biochemist at the Freeman Hospital, Newcastle on Tyne and Dr.G.A.Rose and his team, at St.Pauls Hospital/Institute of Urology in London, the estimation, respectively, of these latter two parameters was carried out for both study group and control urines.

Controls.

Rather than rely on historical Controls it was felt preferable to duplicate all urine collections and analyses exactly, in a group of contemporary Control subjects, in the same laboratory. Clearly it would have been inappropriate for ethical reasons to perform

invasive procedures such as renal biopsy and arterial puncture on control subjects; the source of control renal tissue has been referred to above. Normal blood gas parameters were obtained from the laboratory undertaking the analyses.

44 adults attending the Outpatient Dept. for reasons other than Nephrolithiasis, with no history or clinical evidence of Urinary infection or Renal impairment were asked to complete 24 hour urine collections as above. On receipt of a negative MSU result, the urine was analysed for Calcium, Urate, Oxalate, Citrate and Creatinine after stratification for age and sex.

Urinary Citrate Estimation.

Citric Acid is converted to Oxalo acetate and Acetate in a reaction catalysed by the enzyme Citrate Lyase (CL)

(i) Citrate ----------CL----------> Oxaloacetate

In the presence of the enzymes Malate Dehydrogenase (MDH) and L- Lactate Dehydrogenase (L-LDH), Oxaloacetate and its decarboxylation product, Pyruvate are reduced, respectively, to L-Malate and L-Lactate by reduced nicotinamide-adenine dinucleotide (NADH).

(ii) Oxaloacetate + NADH + H+ ---------> L-Malate + NAD+
MDH

(iii) Pyruvate + NADH + H+ ----------> L-Lactate + NAD+
L-LDH

The amount of LDH oxidised in reactions (ii) and (iii) is stoichiometric with the amount of Citrate. NADH is determined by means of absorbance at 340 nm using the kit manufactured by Boehringer Mannheim GmBH (Cat No. 139076) Analysis was carried out on COBAS B10 centrifugal analyser (manufactured by Roche Products Ltd.)

Sensitivity of the method

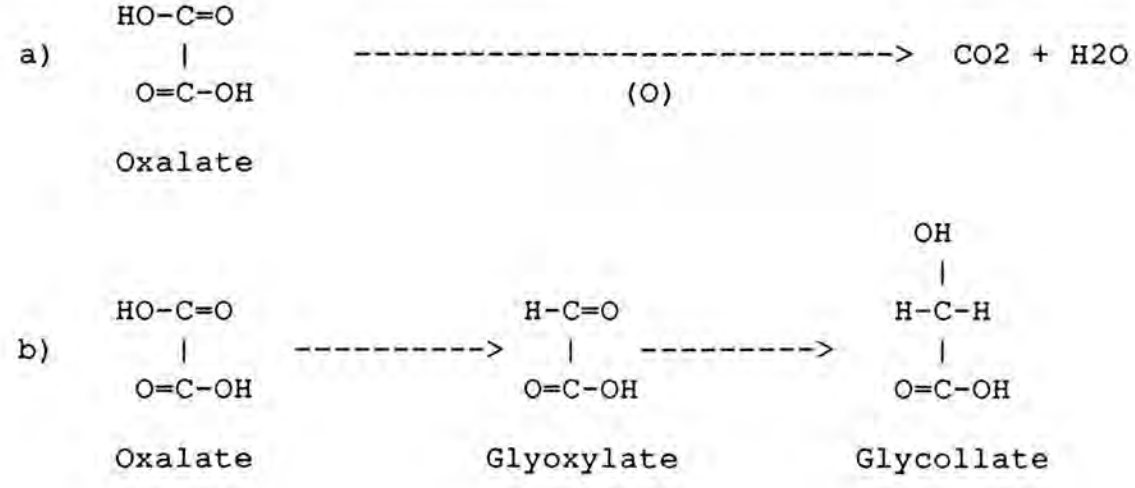
The minimum concentration of Citrate measurable with 95% confidence was 0.2 mmol/L.

Urinary Oxalate Estimation

The main reactions that occur with Oxalate are

a) Oxidation to H2O and CO2 or

b) Reduction to Glycollate via Glyoxylate



Previously fashionable methods can be divided into those requiring separation of the Oxalate from other potentially interfering substances such as Citrate, Magnesium, Ascorbate and Glyoxylate and those using whole urine. In the former group, such preliminary steps included Precipitation, Solvent extraction and Ion exchange chromatography which themselves were complicated by problems of incomplete separation due to contamination, the presence of Inhibitors and lack of specificity.

The subsequent development of methods using whole urine has proved more satisfactory and broadly fall into 3 categories :-

i) Chemical techniques

ii) Physical techniques

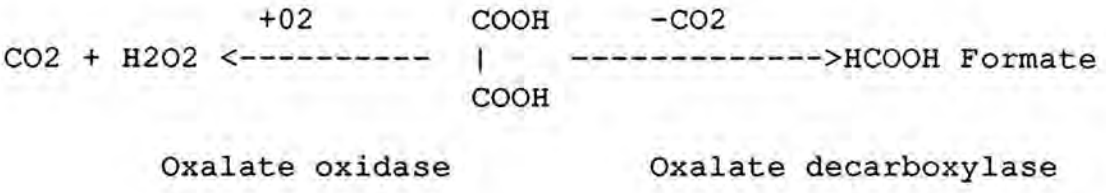
iii) Enzymatic techniques

The chemical method using KMnO4 as an oxidising agent underestimates Oxalate, is not specific and has a low sensitivity. Physical methods using Gas or Ion Chromatography have encountered difficulty with the in vitro conversion of Ascorbic acid to Oxalate during the assay. Enzymatic methods have proved most satisfactory in view of their inherent specificity for Oxalate and because no preliminary separation from urine matrix is required.

Two different enzyme methods have been developed :-

i) Oxalate decarboxylase (Hallson and Rose, 1974)

ii) Oxalate oxidase (Kasidas and Rose, 1985)



The earlier method, was effective but labour intensive, requiring overnight incubation and was not suitable for automation. The advantages of the Oxalate oxidase method include, the use of whole urine, a highly specific and sensitive assay (0.5u mol/L), automation, cost effectiveness (enzyme reusable) and lack of ascorbate interference.

SUMMARY OF RESULTS

Statistical analysis of results was performed using the "OXSTAT" statistical computer software package. Since the groups being examined were unmatched, analyses were performed by the Unpaired t-Test, where data were assumed to be parametric. In cases of doubt regarding this latter assumption, a non-parametric test, the Mann Whitney U test was employed in addition, provided the groups were numerically large enough. Suspected correlation between unrelated groups was calculated and plotted graphically

using the above program and "r" values so obtained matched with standard tables of statistics to assess their significance.

Legend

Abbreviated symbols referring to various patient groups are used throughout the Results section as follows

Male = 1 Female = 2 Control group = K

Calcium = Ca, Urate = Ur, Oxalate = Ox, Citrate = Ct, Creatinine = Cr

e.g. 24hr Calcium excretion, Male stoneformers V Controls

24 Ca 1 | 24 Ca 1 K

Before analysing any of the data, Study subjects and Controls were stratified for Sex, Age and Body mass, (24hr Urinary Creatinine being used as an approximate estimate of body mass, given normal renal function.) Renal function was assumed to be grossly normal in the presence of serum Creatinine or Urea values within the laboratory reference range.

DATA

Age (Years)		Mean		S.D.
	Males	50.48	S.D.	11.92
	Controls	51.20	S.D.	13.73
	Females	49.34	S.D.	15.48
	Controls	44.17	S.D.	17.22

(81 - 2)

There was no significant difference between Males and Controls or Females and Controls. Where the apparent slight difference in Age between Females and Controls might be thought to affect results, a more critical breakdown of age pattern was undertaken. (See Citrate analysis and Discussion)

Body mass (Urinary Creatinine m mol/24hr)

Males	13.559	S.D.	4.437
Controls	11.632	S.D.	3.638
Females	9.192	S.D.	2.856
Controls	9.491	S.D.	1.941

(86 - 7)

There was no significant difference between Males and Controls or Females and Controls.

As well as calculating 24hr excretion of a given substance (S), in order to avoid any error as a result of inherent 'body mass effect', the ratio (S)/Creatinine was also calculated.

Renal Function		Mean	S.D.	N
Serum Urea	(males)	5.418	1.399	54
	(females)	5.181	1.487	26
(Normal lab. values 2.5 - 6.6 m mol/L)				
Serum Creatinine	(males)	103.375	16.122	32
	(females)	82.000	13.260	13
(Normal Lab. values 55 - 150 u mol/L) (83)				

Serum Calcium

	(males)	2.344	0.105	54
	(females)	2.398	0.118	24
(Normal Lab. values 2.12 - 2.62 m mol/L)				

Serum Urate

	(males)	0.347	0.089	50
	(females)	0.347	0.075	23
(Normal Lab values mmol/L)		0.12 - 0.36		Females
		0.12 - 0.42		Males

(83)

24Hr Urine Volume (L)

Males	2.02	S.D. 0.65	
Controls	1.45	S.D. 0.57	$p < 0.001$
Females	1.80	S.D. 0.81	
Controls	1.43	S.D. 0.60	$p = 0.08$

(84-5)

The difference between Males and Controls was significant, however, the significance of this finding is less certain. It may simply reflect the compliance with medical advice to increase fluid intake.

Renal Biopsy - Microcalcification

Males Microcalcification present 28/48 = 58.3%

Females Microcalcification present 13/21 = 61.9%

Controls Microcalcification present 7/30 = 23.3%

The biopsies included on average 18 glomeruli (Range 2-50)

Calcium was seen in approx. 60% of cases (Male = Female) the majority occurring in the Medulla.

Renal Biopsy - Pathological abnormalities

In addition to calcification, a number of other abnormalities was shown on biopsy |- Chronic Interstitial Nephritis (an increase in interstitial cells by subjective assessment), Fibrosis shown by MSB preparations, Tubular Atrophy and Glomerular Hyalinisation indicating permanent renal damage and a few examples of Acute Tubular Necrosis and Mesangial Proliferation.

Overall there appeared to be an association between the presence of Calcification and other Pathology, Calcium being present in 70% of cases where a pathological abnormality was seen and in 35% of those where histology was normal.

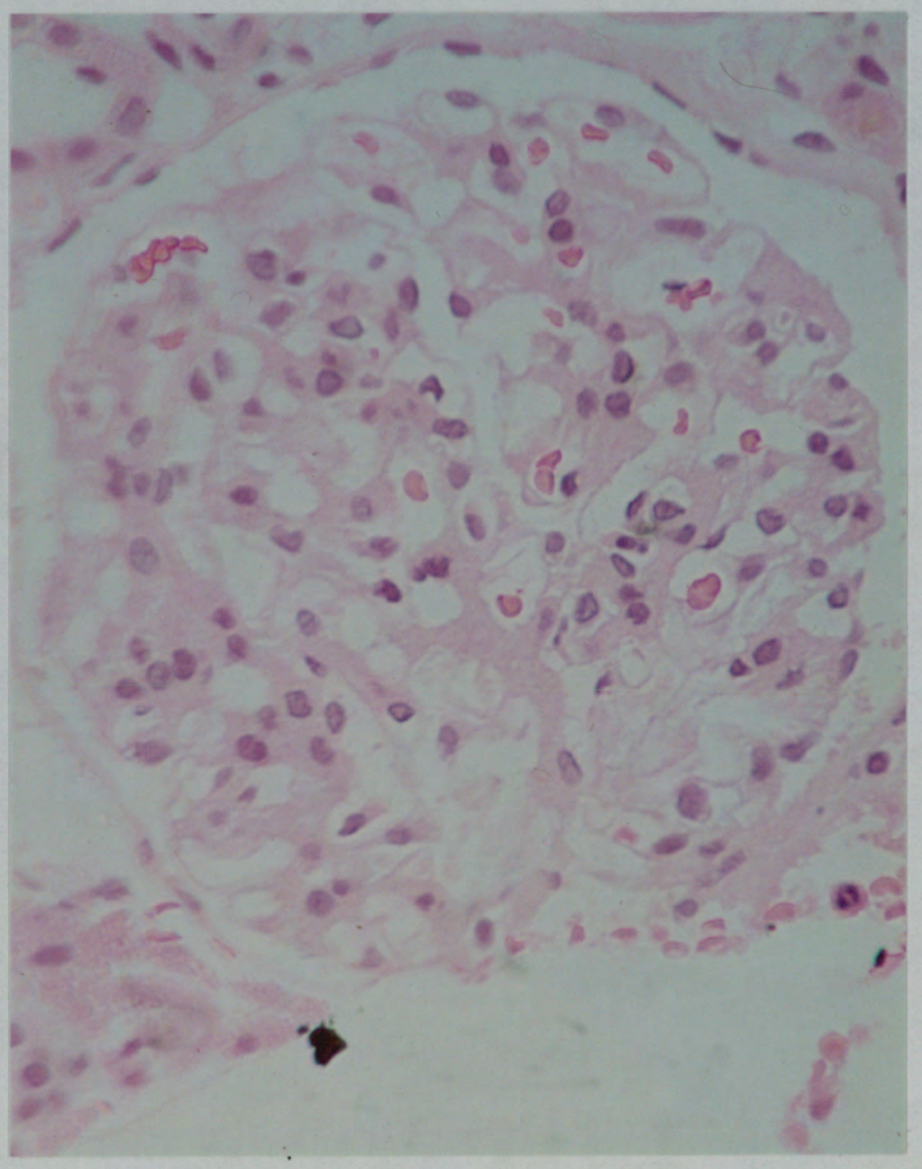
Examination of the Data on Renal biopsy Calcification compared with Urinary excretion of Calcium, Urate, and Oxalate showed no significant difference between those with and without Calcium on biopsy. Similar data relating Citrate excretion to biopsy appearance shows a statistically significant difference in Males ($p = 0.014$) paradoxically the group with Calcification on biopsy having the higher Citrate excretion. Conversely, in Females, while the Mean Citrate excretion is lower in the group with microcalcification, this does not reach statistical significance ($p = 0.18$), perhaps reflecting the very small size of this subdivided group.

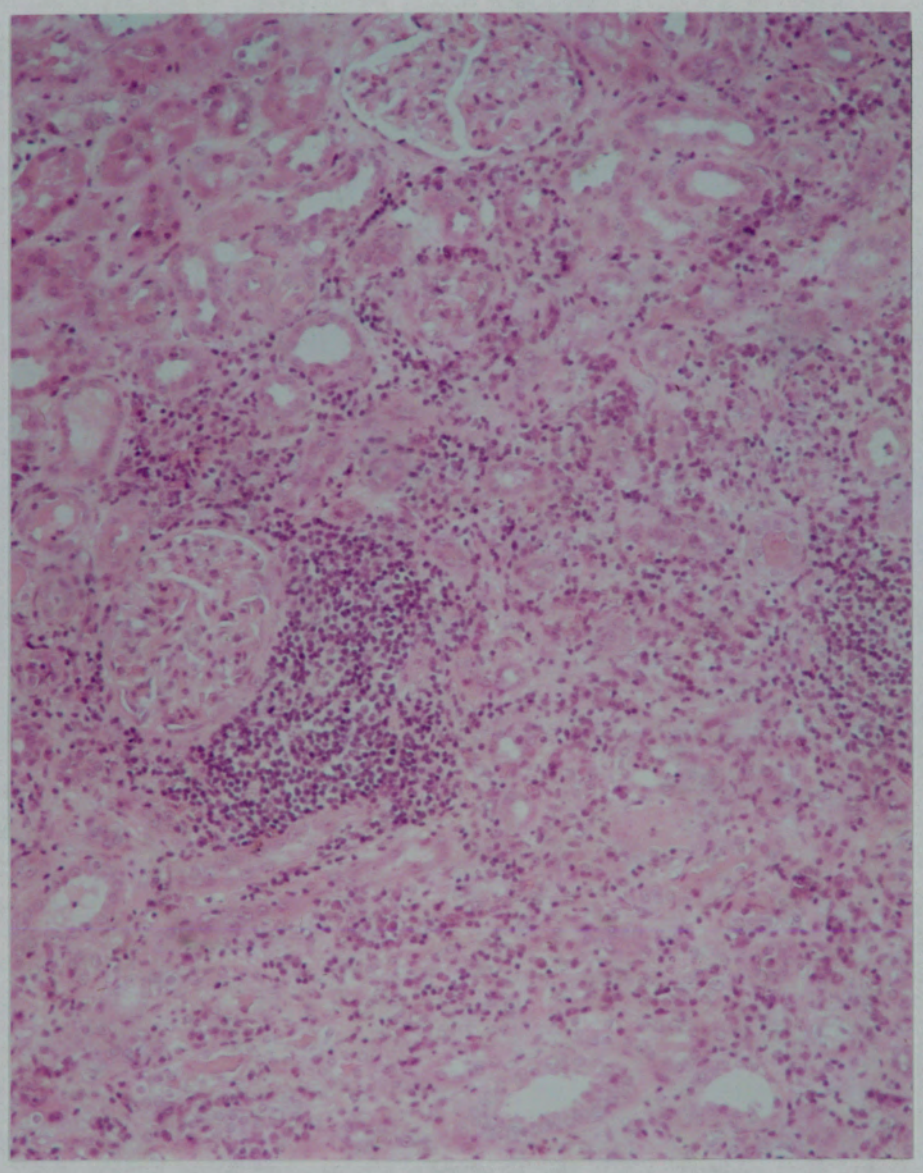
RENAL BIOPSIES IN STONEFORMERS

Photographic prints by kind permission of Dr.D.J.Harrison
Dept. of Pathology, University of Edinburgh

NORMAL GLOMERULUS

(H & E)





Chronic Interstitial

Nephritis with

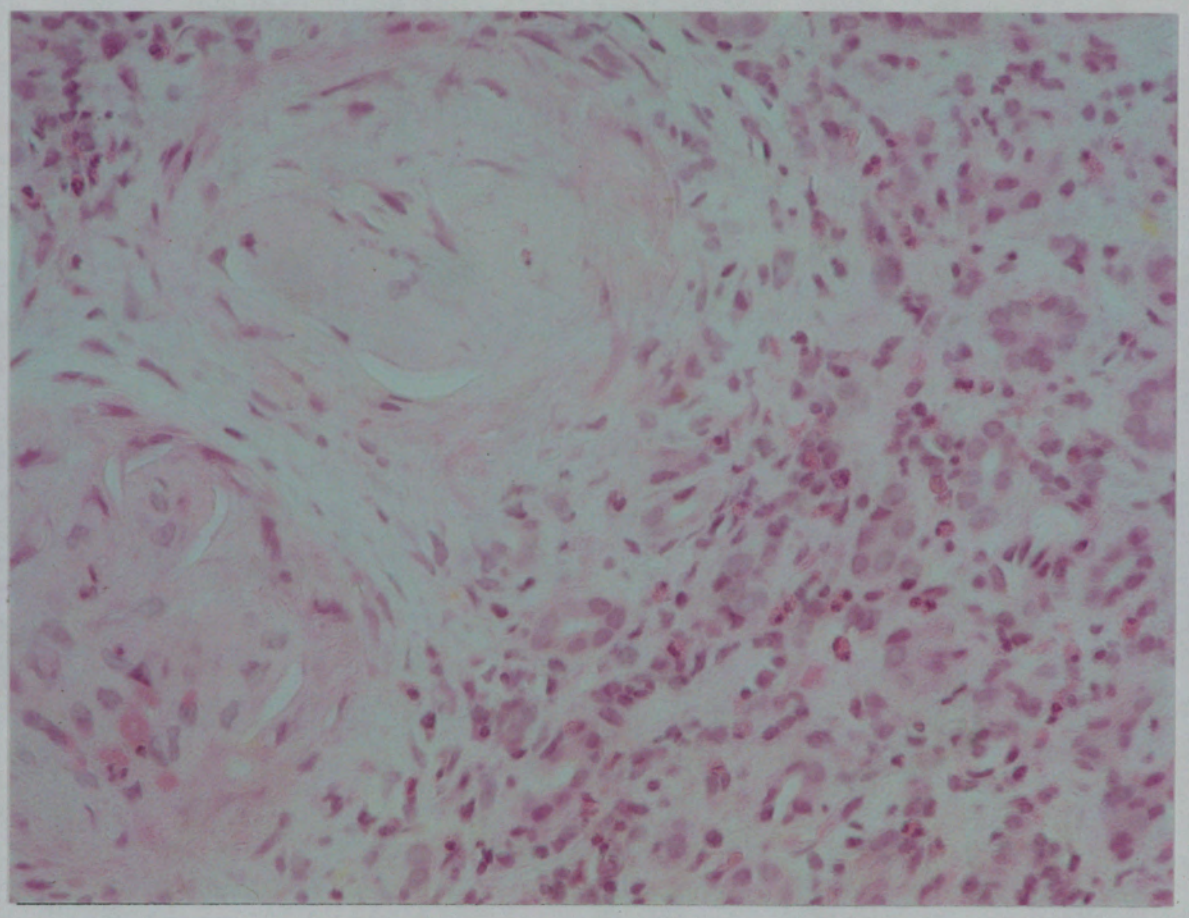
Tubular Atrophy

(H & E)

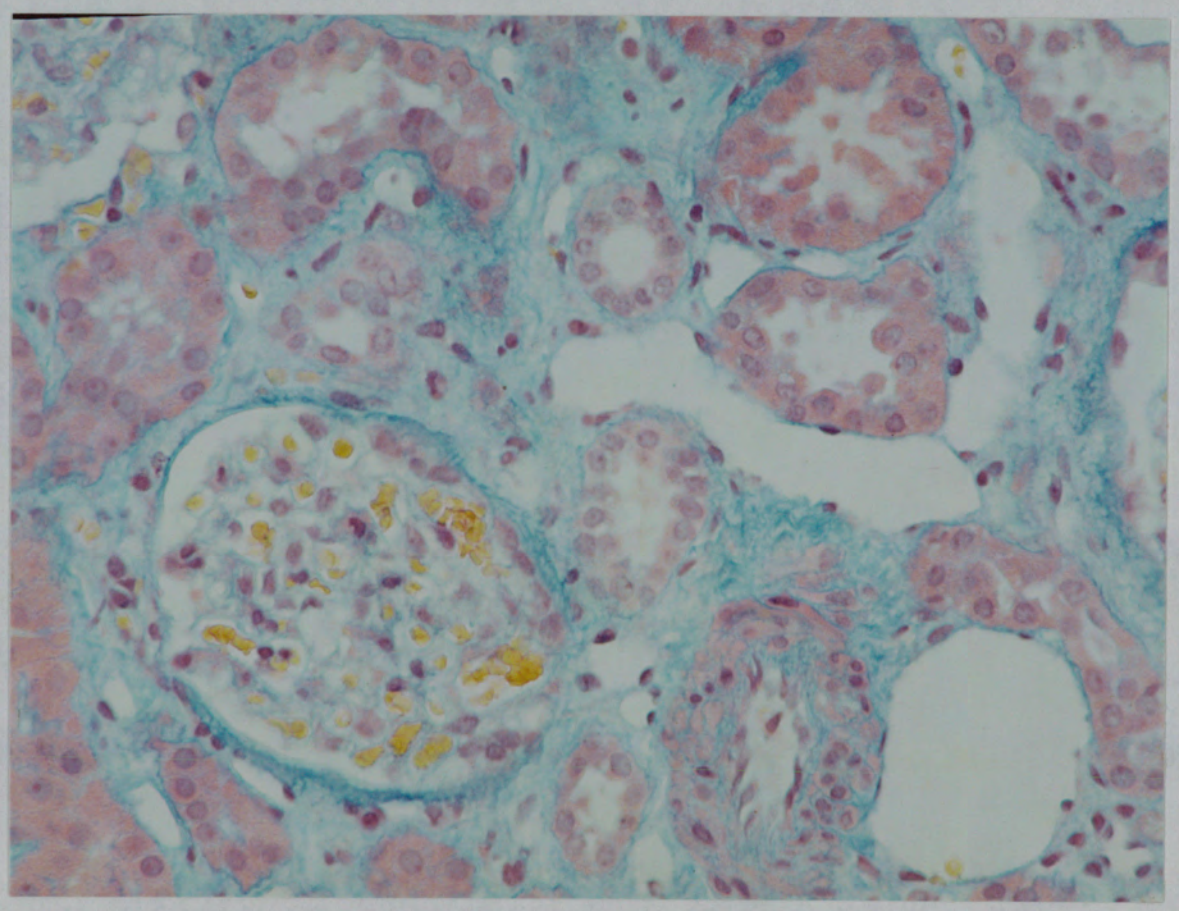
Legend:

Glomerulus

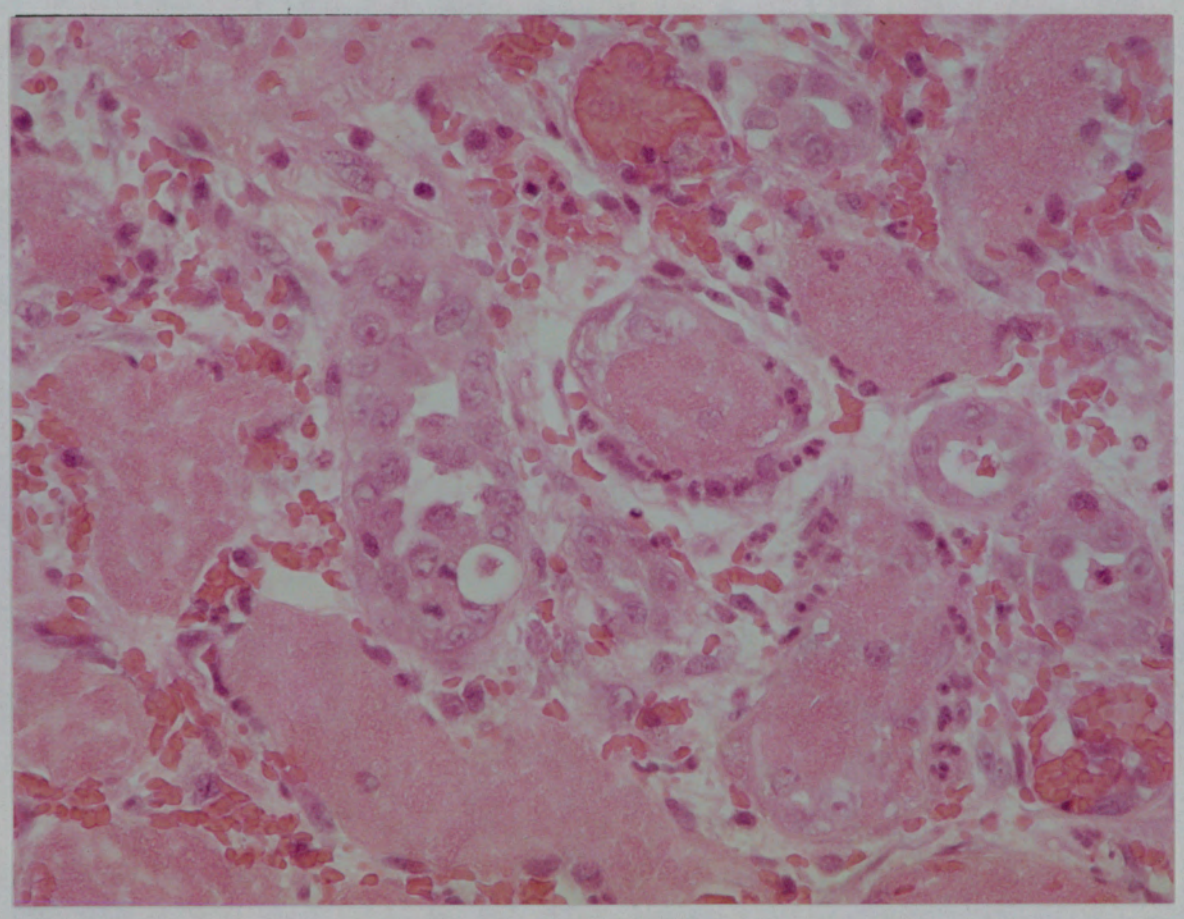
Lymphocytes



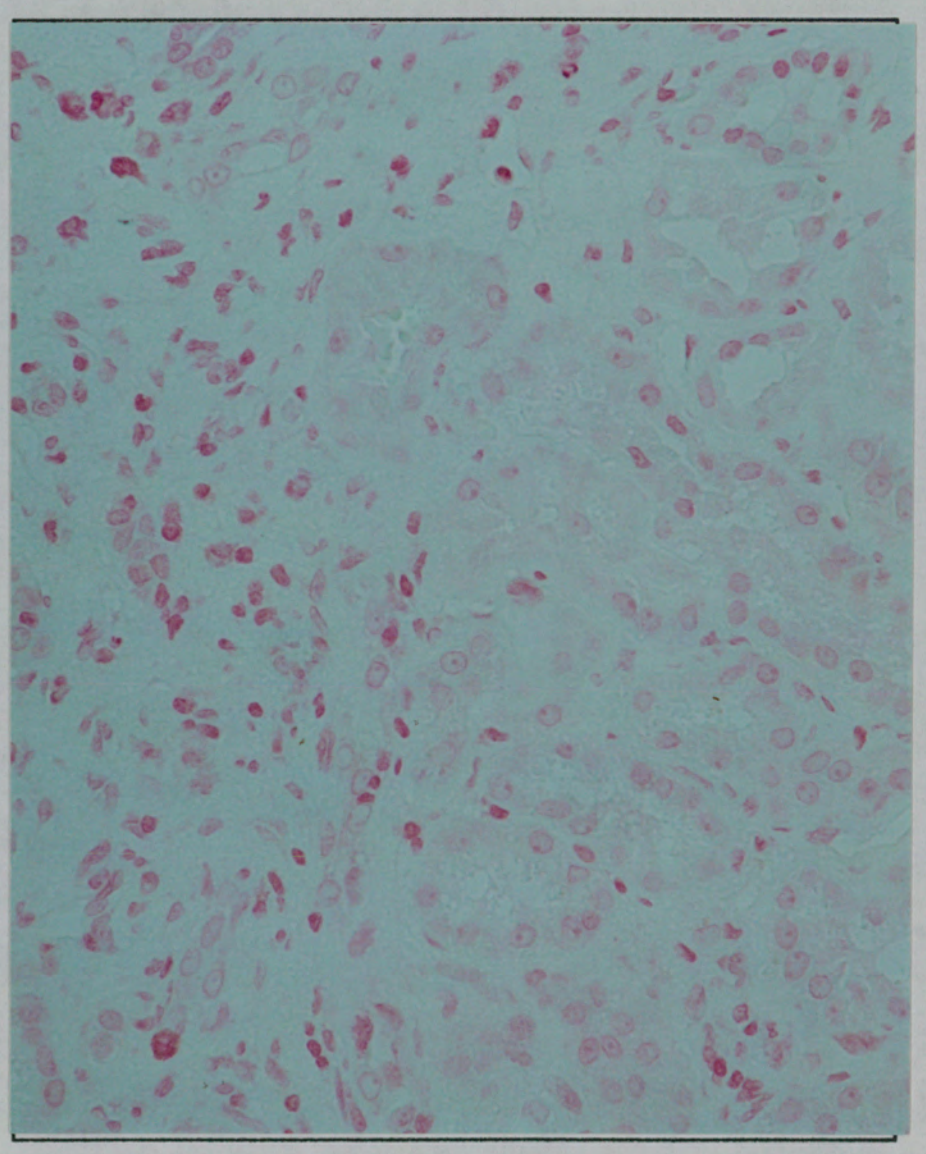
Interstitial Fibrosis
Acute Inflammation
and Tubular Atrophy
Two Hyalinised
Glomeruli shown
(H & E)



Interstitial Fibrosis
(Martius Scarlet)
(Blue)



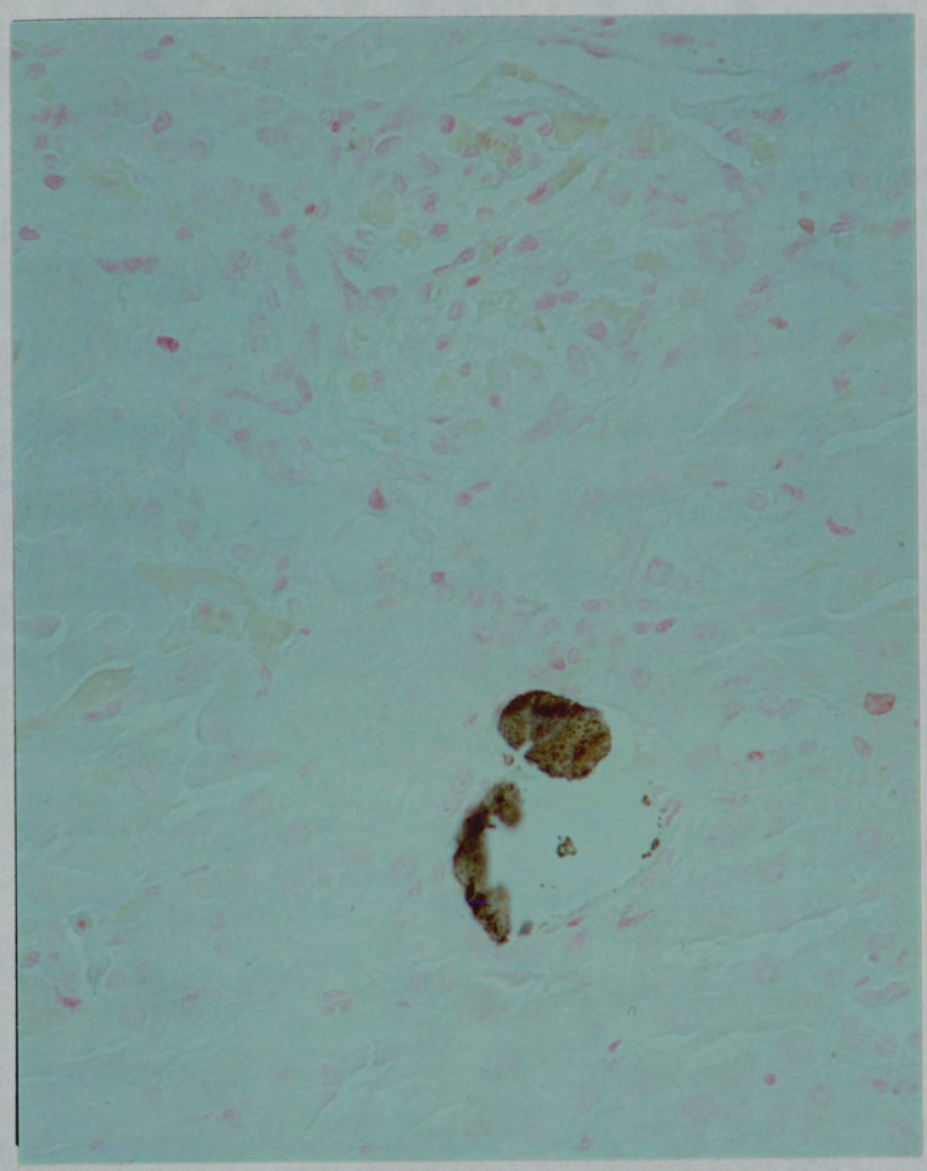
Acute Tubular Necrosis showing
Congestion and Tubulorrhexis
(H & E)



Renal Biopsy

NEGATIVE for Calcium

(von Kossa)



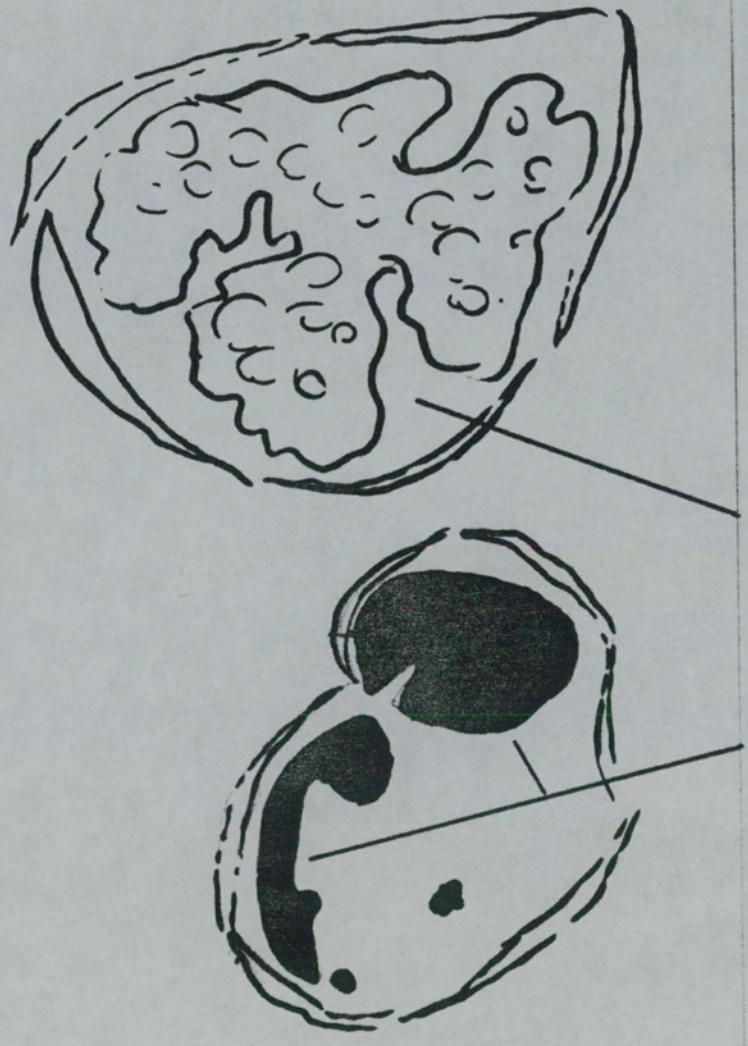
Renal Biopsy -

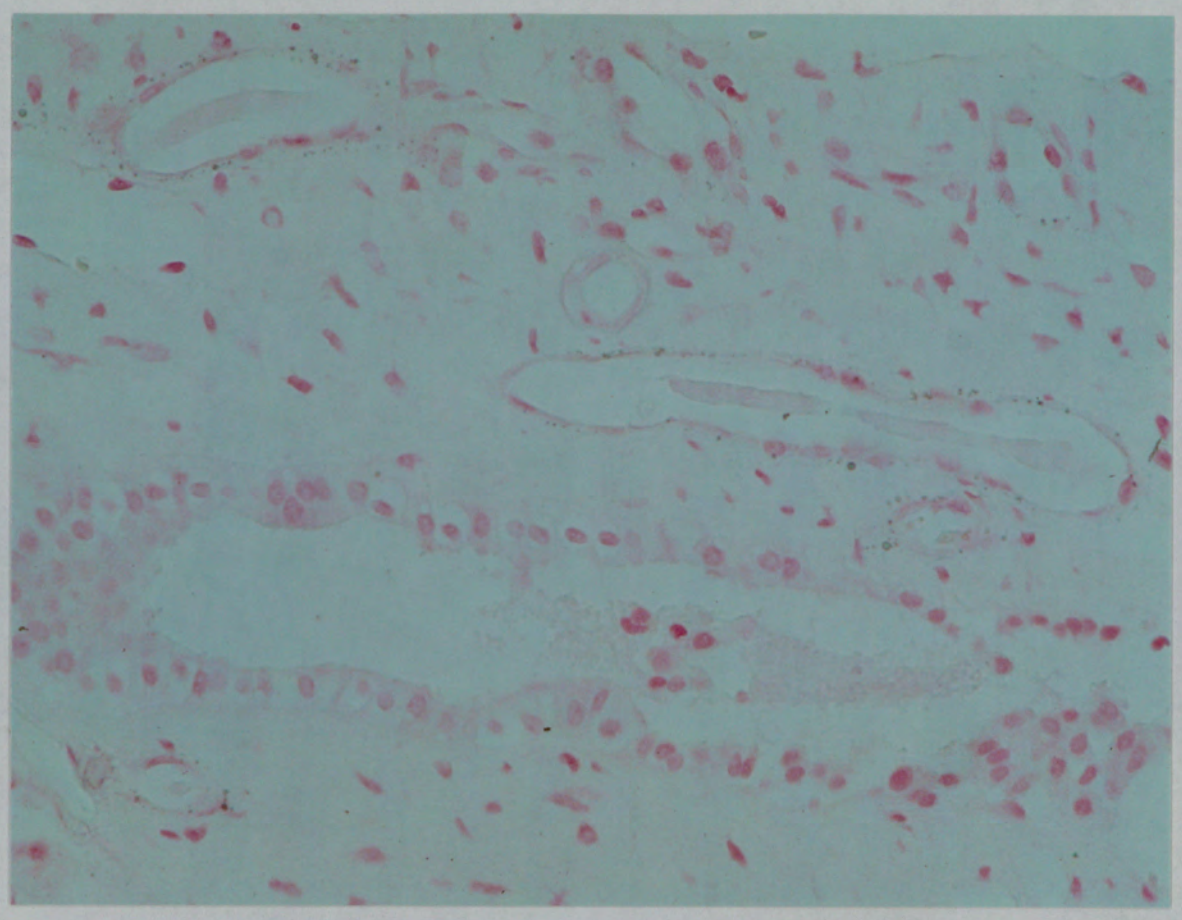
Calcified Deposit

in Lumen of

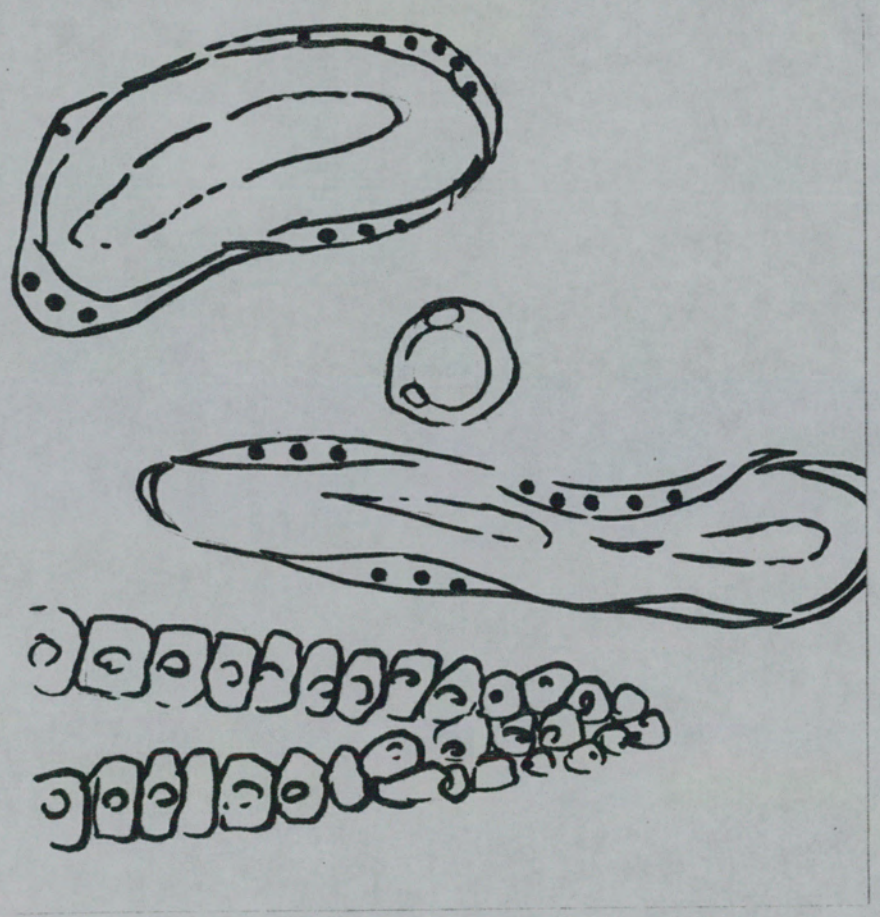
Cortical Tubule

(von Kossa)

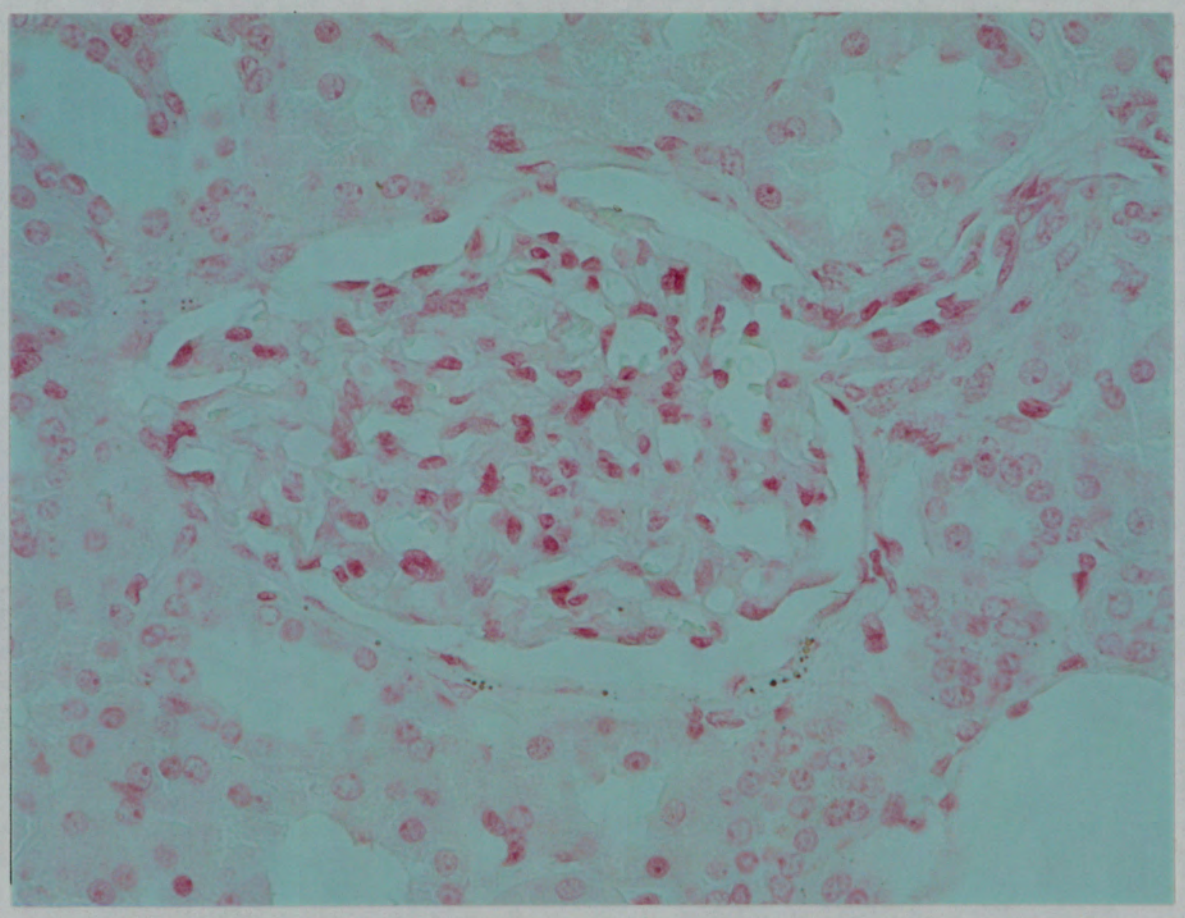




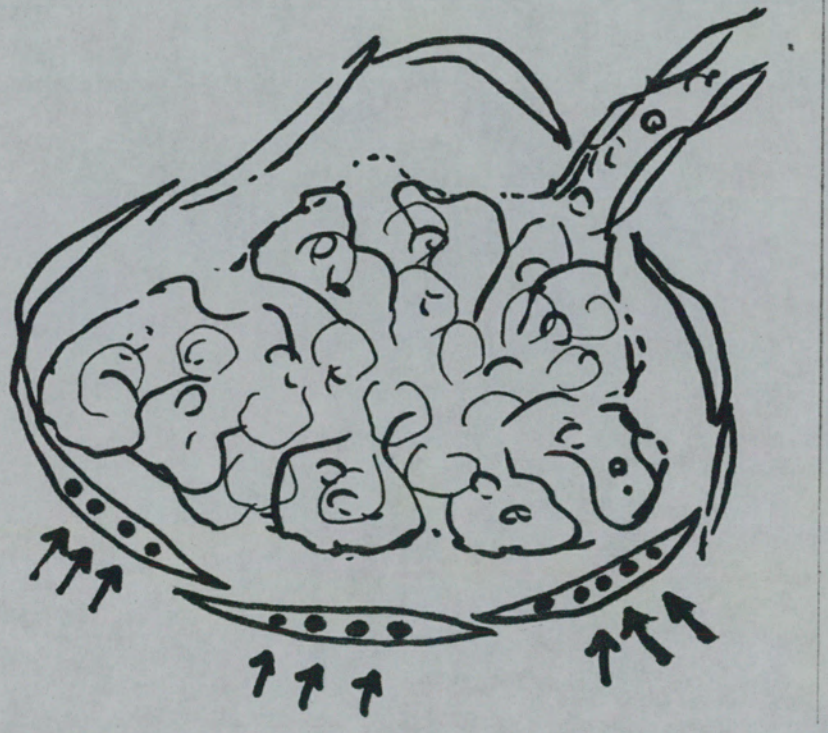
Microcalcification in Basement Membrane of
Medullary Tubule (von Kossa)



Legend: Microcalcification (•••)

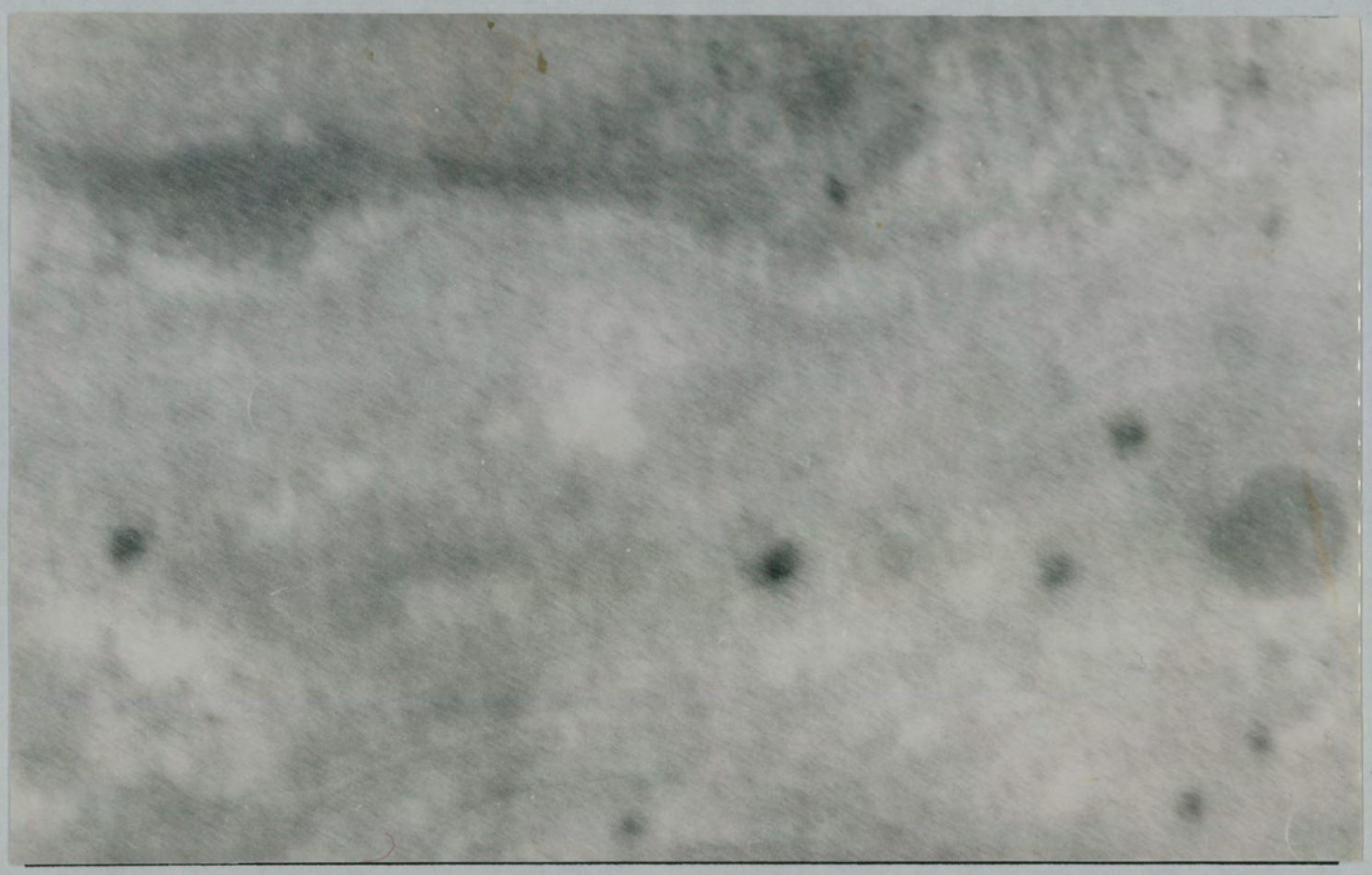


Microcalcification in Bowman's Capsule
of Glomerulus (von Kossa)

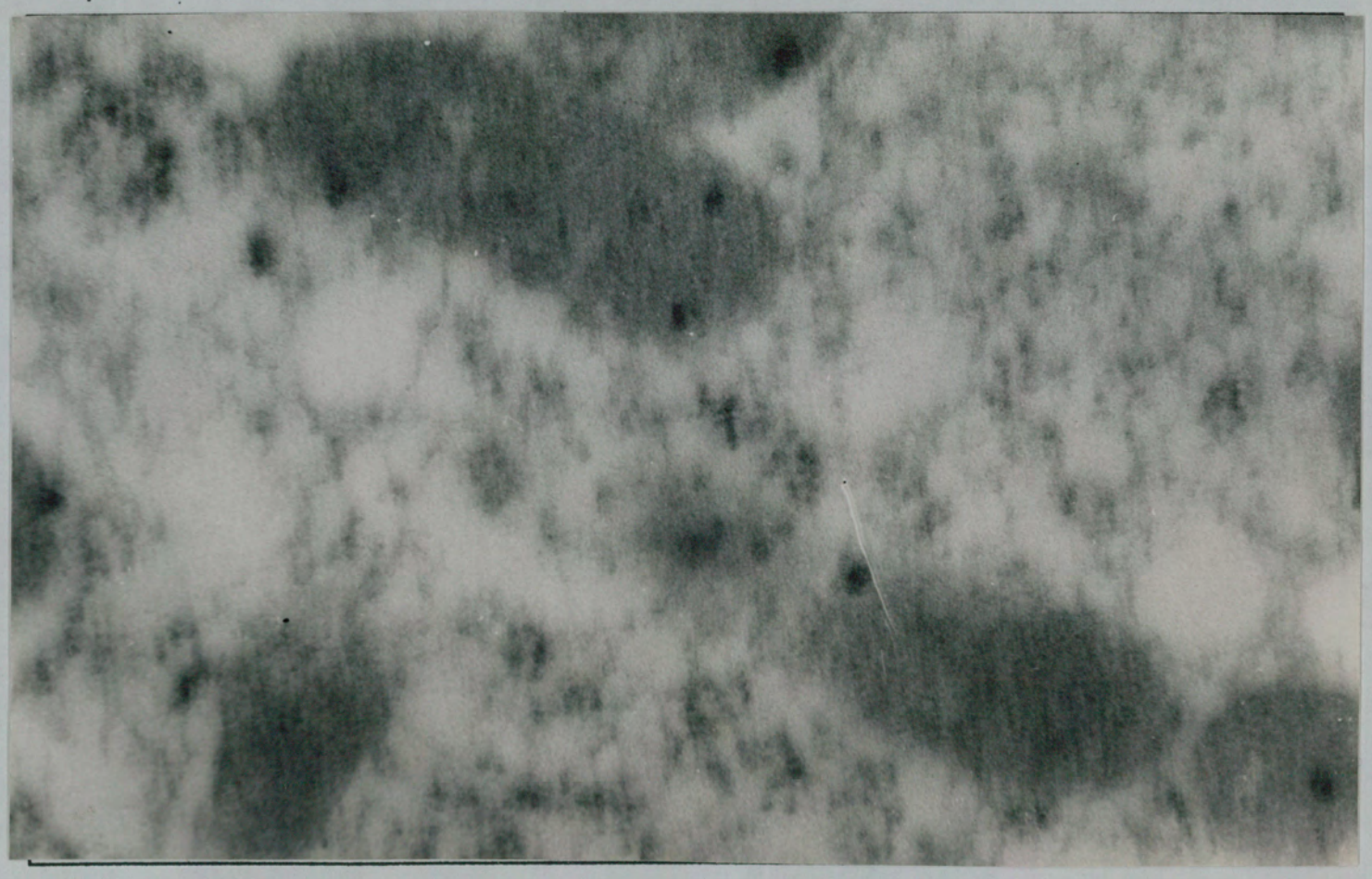


Legend: Calcification (arrowed)

RENAL BIOPSIES - ELECTRON MICROGRAPHS



i) CALCIUM DEPOSITS IN TUBULAR BASEMENT MEMBRANES



ii) CALCIUM DEPOSITS WITHIN MITOCHONDRIA

RELATIONSHIP OF CALCIFICATION AND RENAL BIOPSY SITE

IN STONEFORMERS

TISSUE:	CORTICO/MEDULLARY JNCT.		MEDULLA		CALYX	
MALES (48)						
Calcification	+	-	+	-	+	-
Number of Cases	16	14	10	1	2	5
FEMALES (21)						
Calcification	+	-	+	-	+	-
Number of Cases	8	4	1	2	4	2

INCIDENCE OF CALCIFICATION AT CORTICO/MEDULLARY JUNCTION	24/42	57.1%
INCIDENCE OF CALCIFICATION AT MEDULLA	11/14	78.6%
INCIDENCE OF CALCIFICATION IN RENAL BIOPSY (MALES)	28/48	58.3%
INCIDENCE OF CALCIFICATION IN RENAL BIOPSY (FEMALES)	13/21	61.9%

RELATIONSHIP BETWEEN PATHOLOGICAL ABNORMALITY AND CALCIFICATION IN RENAL BIOPSIES OF STONEFORMERS

HISTOLOGY:	NORMAL		INTERSTITIAL NEPHRITIS		INTERSTITIAL FIBROSIS	
MALES (48)						
Calcification	+	-	+	-	+	-
Number of Cases	6	8	13	7	6	2

MESANG.PROLIF.		DYSPLASIA		ADENOMA		N/AVAILABLE	
+	-	+	-	+	-	+	-
2	1	0	1	1	0	0	1

HISTOLOGY	NORMAL		INTERSTITIAL NEPHRITIS		INTERSTITIAL FIBROSIS	
FEMALES (21)						
Calcification	+	-	+	-	+	-
Number of Cases	1	5	11	2	0	0

MESANG.PROLIF.		DYSPLASIA		ADENOMA		N/AVAILABLE	
+	-	+	-	+	-	+	-
0	1	0	0	0	0	1	0

	MALES	FEMALES
INCIDENCE OF PATHOLOGICAL ABNORMALITY	70.2% (33/47)	70.0% (14/20)
INCIDENCE OF CALCIFICATION IN THOSE WITH PATHOLOGICAL ABNORMALITY	66% (22/33)	78.6% (11/14)
INCIDENCE OF CALCIFICATION IN THOSE WITH NORMAL HISTOLOGY	42.8% (6/14)	16.6% (1/6)
INCIDENCE OF CALCIFICATION IN RENAL BIOPSY	58.3% (28/48)	61.9% (13/21)

URINE CHEMISTRY RESULTS

Urinary Calcium Excretion (m mol / 24hr)

Males	8.131	S.D. 3.304	
Controls	4.784	S.D. 2.827	$p < 0.001$
Calcium / Creatinine ratio			$p < 0.001$

(92 - 4)

The frequency distribution seems Gaussian for standard Data however the data referring to Ca/Cr ratios are positively skewed. Both para- and non-parametric testing show highly significant differences between groups. In 20% of Male stoneformers urinary Ca was greater than Mean (Control) + 2 S.D.

Females	5.916	S.D. 3.422	
Controls	2.855	S.D. 1.560	$p < 0.001$
Calcium/Cr Ratio			$p < 0.005$

The frequency distribution was non-Gaussian. Both para- and non-parametric tests show highly significant differences between groups. In 39% of Female stoneformers Urinary Calcium was greater than Mean Control) + 2 S.D.

(95 - 7)

Urinary Urate Excretion (m mol / 24hr)

Males	4.539	S.D. 1.499	
Controls	2.712	S.D. 1.429	$p < 0.001$
Urate / Cr Ratio			$p < 0.01$

Stoneformers and Controls had a Gaussian distribution. The reduced significance of Urate/Cr ratio may reflect an underlying Mass effect. In 19.6% of Male stoneformers urinary Urate was greater than Mean (Control) + 2 S.D.

Females	3.404	S.D. 1.305	
Controls	3.428	S.D. 1.494	p > 0.95
Urate/Cr ratio			p = 0.508

There was no significant difference in Urate excretion between Female stoneformers and Controls.

(98 - 103)

Urinary Citrate Excretion (m mol / 24hr) (110 - 14)

Males	2.384	S.D. 1.332	
Controls	2.459	S.D. 1.663	p > 0.8
Citrate / Cr Ratio			p > 0.6

There was no significant difference in the groups using either parametric or non-parametric methods, the frequency distribution in both Male stoneformers and Controls showing a non-Gaussian pattern with a slight positive skew.

Females	2.565	S.D. 2.054	
Controls	4.078	S.D. 2.689	p = 0.056
Citrate / Cr. ratio			p = 0.027

There is a significant difference between Female stoneformers and Controls. The frequency distribution however shows a non-Gaussian picture there being a skew to the Left in both stoneformers and Controls. There is also a suggestion that this group comprises two separate populations.

Plotting this Data, (Female Citrate/Cr.Ratio v Controls), reflects this skewed distribution and shows that no stoneformers in fact fall out of the range (Control Mean - 2 S.D.) (127)

Citrate Excretion (Citrate/Cr Ratio) (116)

Male Controls	0.206	S.D. 0.113	
Female Controls	0.440	S.D. 0.278	$p < 0.001$

The Control values in Female subjects are significantly higher than in Males, as has been shown in other studies reflecting a presumed Oestrogen effect in pre-menopausal women. As the female stoneformers in this study form a marginally older population than the female Control group, it could be argued that the difference in observed Citrate excretion in this group reflects age related hormonal effects rather than any other cause. To investigate this possibility, further stratification of stoneformer and Control groups by Age, into pre- and postmenopausal sub-groups (<50 yrs and > 50 yrs.) was

undertaken. It can be seen that the previously noted difference between female stoneformers and controls is preserved and therefore cannot be an Age related phenomenon.

Citrate Excretion (Female Stoneformers)			(129)
≥ 50yrs	2.79	S.D. 1.80	
< 50 yrs	2.36	S.D. 2.33	p = 0.65

Citrate Excretion (Female Controls)			
≥ 50 yrs	4.25	S.D. 2.20	
< 50 yrs	3.96	S.D. 3.07	p = 0.80

Exploring the hypothesis suggested by the frequency distribution histogram for Citrate excretion in female stoneformers that there were 2 separate populations within the stoneforming group, the Data for Citrate excretion were re-examined after exclusion of patients (and Controls) with abnormal excretion of Calcium or Urate or Oxalate. "Normal" was defined as within the range, (Control Mean + 2 S.D.).

Revised Citrate Excretion (Excluding patients with Calcium, Urate or Oxalate > Control Mean + 2 S.D.)			
Males	2.374	S.D. 1.342	
Controls	2.281	S.D. 1.587	p = 0.669
Citrate/Cr ratio			p = 0.318

Revised Citrate Excretion (Excluding patients with Calcium, Urate or Oxalate > Control Mean + 2 S.D.)

Females	1.768	S.D. 1.490	
Controls	4.354	S.D. 2.625	p = 0.002
Citrate/Cr ratio			p = 0.004

By excluding patients with high levels of Calcium, Urate or Oxalate the difference between Stoneformers and Controls becomes much more significant.

(123 - 6)

Urinary Oxalate Excretion (m mol / 24hr)

Males	0.213	S.D. 0.117	
Controls	0.228	S.D. 0.114	p = 0.317
Oxalate / Cr Ratio			p = 0.125

Females	0.184	S.D. 0.084	
Controls	0.230	S.D. 0.134	p = 0.412
Oxalate / Cr ratio			p = 0.295

There was no detectable difference in Oxalate excretion between stoneformers and Controls of either sex.

(104 - 109)

STONE FORMATION INDEX

An attempt was made to devise a numerical index of Lithogenicity based on various products of the excretion Data presented above. No index, however, proved any better a discriminant between Stoneformers and Controls than the Calcium/Creatinine ratio alone.

The product $\frac{\text{Calcium x Urate}}{\text{Citrate x Creatinine}}$ which might be expected to exaggerate any difference between stoneformers and Controls, showed the same significance as simple urine Calcium/Cr ratios alone.

Male Stoneformers	v	Controls	$p < 0.001$
Female Stoneformers	v	Controls	$p = 0.004$

(130 - 4)

Coefficient of Correlation

There was no significant correlation between 24hr Calcium excretion and 24 hr Creatinine excretion in Male or Female Stoneformers although there was some positive correlation in Male Controls ($p = 0.015$).

(135 - 6)

There was no correlation between Urate excretion and Creatinine excretion in Males however in Females there was significant correlation.(Stoneformers $p = 0.069$ and Controls $p < 0.001$)

As there was no observed difference between Female subject and Control Urate excretion this was considered insignificant. (137 -39)

Citrate excretion in Male stoneformers was not correlated with Creatinine excretion, however, in Females there was a significant correlation ($p = 0.003$). As there was no difference in Creatinine excretion between Female stoneformers and Controls, this should not affect any conclusions drawn regarding Citrate excretion in Females. (140 – 2)

There was no correlation between Calcium and Urate excretion in Controls, however, in both Male ($p = 0.011$) and Female ($p < 0.001$) stoneformers there was an association which was significant (and which persisted when Urate/Cr and Calcium/Cr ratios were plotted. ($p < 0.001$, $p = 0.034$)

This suggests that the observed difference in excretion of Calcium and Urate in stoneformers compared with Controls may not be independent variables. (143 – 7)

There was no correlation between Calcium and Citrate excretion in Male stoneformers and the correlation observed in Male Controls was no longer significant when Creatinine ratios were examined.

In Female stoneformers significant correlation was demonstrated ($p < 0.001$) and was only slightly reduced in significance when Citrate/Cr ratios were calculated, ($p = 0.007$). This finding could suggest a possibly protective increase in Citrate with increasing hypercalciuria in Female stoneformers. Another explanation might be that Calcium and Citrate were complexed together in the urine as suggested by Menon (1983) who observed the same association.

(148 – 52)

There was no correlation between Citrate and Urate excretion. (153 – 56)

In order to test the hypothesis that stone formers with low Citrate excretion might have incomplete distal RTA as an underlying cause, arterial blood gas analysis was undertaken on all consenting stoneformers (but not in Controls) in order to detect any degree of Metabolic acidosis which might be present. Arterial Bicarbonate in the stoneformer group as a whole was not outside the laboratory reference range.

art. HCO3	Male	23.08	S.D. 1.82
	Female	24.08	S.D. 1.82

(ref. range 21 - 27.5 m mol / L)

(157)

When Coefficient of Correlation was examined combining Data on arterial HCO3 and Citrate/Creatinine ratios, while there was no correlation found in Male stoneformers, there was a positive correlation in Female stoneformers which was significant ($p = 0.031$). This observed association of low arterial Bicarbonate and low Citrate Excretion in Females is consistent with a degree underlying Renal Tubular Acidosis in a proportion of this group of Stoneformers. (161 - 64)

Plotting of individual data points, however, (Female Citrate/Creatinine | Controls) shows no stoneformers outwith the range of Control values (Mean - 2 S.D.) as a result of the positively skewed distribution.

(127)

In an attempt to clarify this finding, data referring to Citrate excretion in Females (for both Stoneformers and Controls) was recalculated after deletion of all values of Citrate/Cr < 0.16. in case the group of samples with apparently very low Citrate excretion had been contaminated after collection or infected in vivo and were therefore spuriously low. In total, 8/21 (38%) of female stoneformer group and 4/20 (20%) of Controls were thus deleted.

Subsequent parametric testing of remaining Data showed a persisting significant difference between the groups, ($p = 0.06$). There were insufficient remaining Data to perform the non-parametric Mann Whitney U test.

When the reduced data on Citrate/Cr Ratios (Having removed low Citrate values ?? RTA Patients) was once more correlated with arterial HCO3 data the previously observed significant association was absent. This would suggest that the low Citrate excretion group which was deleted also included the slightly acidotic individuals revealed by Arterial gas analysis and that this group indeed have a degree of hypocitraturic acidosis (?RTA) and have not simply been subject to sample contamination or infection. (117–8, 165)

We showed (above) that Data comparing Citrate excretion in Female stoneformers with Controls were more significantly different after excluding patients with abnormally high excretion of Calcium, Urate or Oxalate.

When these revised Data are correlated with Arterial HCO3 again a positive correlation is shown, $r = 0.654$ however the group size is small ($N = 8$), and statistical significance is marginal, ($p = 0.079$).

Similar calculations in all the above groups comparing H+ instead of HCO3 shows no correlation: this would be consistent with a degree of chronic compensated acidosis rather than an acute phenomenon.

(158–60)

Correlation of Citrate/Creatinine ratios with Serum Creatinine data (equiv. to Renal Function) shows the expected negative correlation of Urinary Citrate with impaired renal function in female stoneformers ($p = 0.05$) but this is not evident in Males. (170 – 73)

As our study population had renal function within the normal reference range this observation should not affect our conclusions regarding Citrate Excretion and in particular does not necessarily mean that Citrate excretion in female stoneformers is low secondary to impaired renal function. In fact serum Creatinine was higher, overall, in male stoneformers yet no such negative correlation between Citrate excretion and serum Creatinine was observed.

(169)

COMPREHENSIVE DATA AND STATISTICAL ANALYSIS OF RESULTS

AGE DETAILS (YEARS) :- MALES (1)
MALES CONTROLS (1K)
FEMALES (2)
FEMALES CONTROLS (2K)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
2	AGE 1	50.48	11.92	1.57	58
3	AGE 1 K	51.20	13.73	3.07	20

F ratio = 1.3259 f1 = 19 f2 = 57 p >=0.1

Assuming Equal Variance : T = 0.2231 DF = 76 p = 0.824

Assuming Unequal Variance : T = 0.2082 DF = 30 p = 0.836

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
4	AGE 2	49.34	15.48	2.87	29
5	AGE 2 K	44.17	17.22	3.51	24

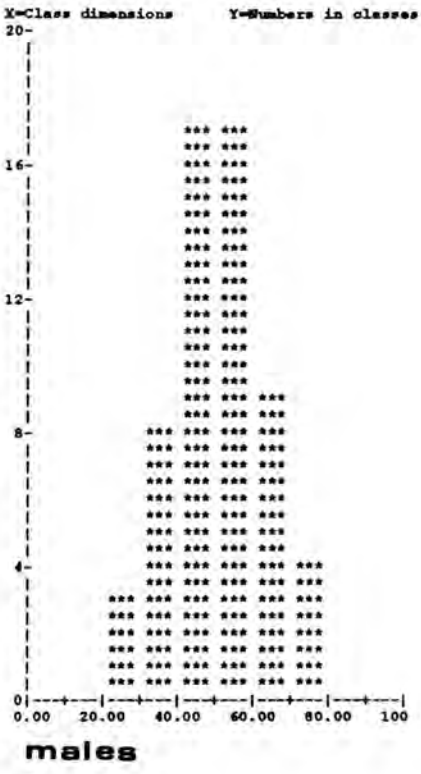
F ratio = 1.2368 f1 = 23 f2 = 28 p >=0.1

Assuming Equal Variance : T = 1.1522 DF = 51 p = 0.255

Assuming Unequal Variance : T = 1.1405 DF = 47 p = 0.260

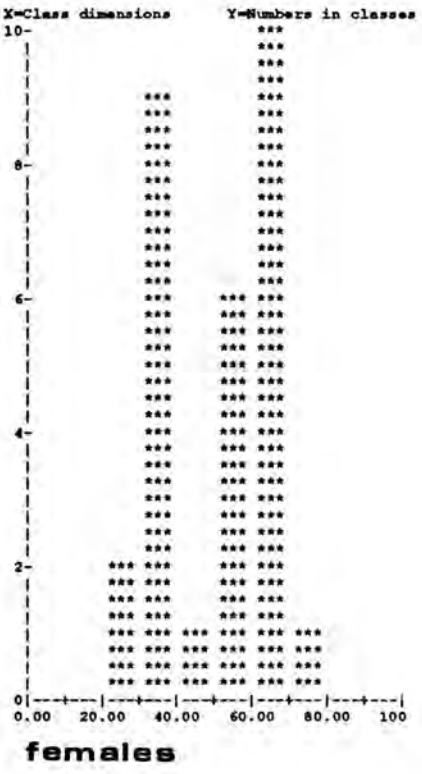
AGE DETAILS (FREQUENCY DISTRIBUTIONS)

Data from columns 2 to 2 and rows 1 to 134



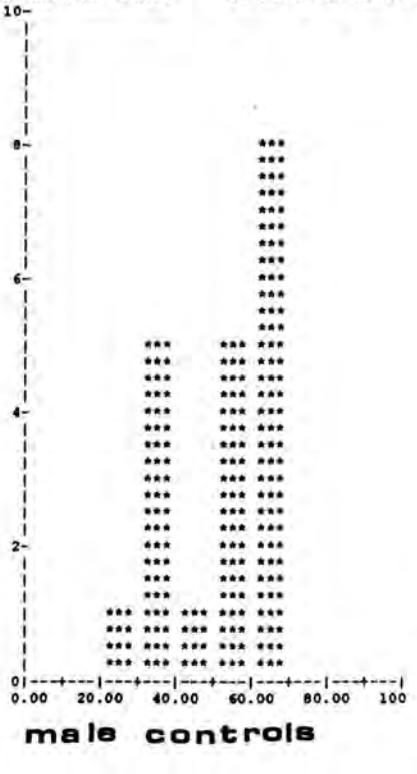
males

Data from columns 4 to 4 and rows 1 to 134



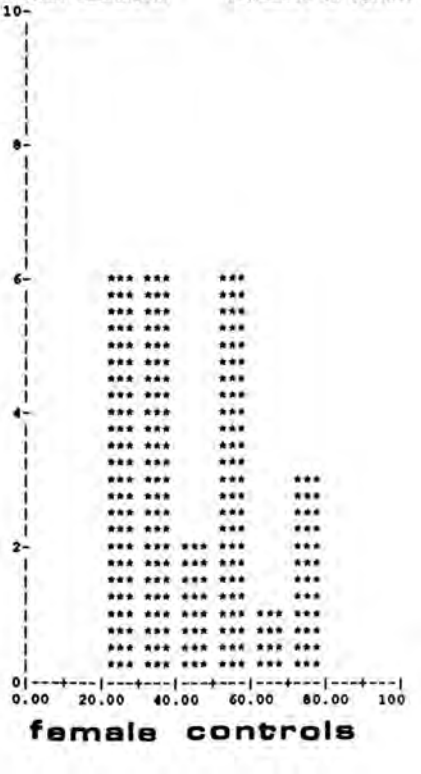
females

Data from columns 3 to 3 and rows 1 to 134



male controls

Data from columns 5 to 5 and rows 1 to 134



female controls

SERUM CALCIUM, URATE, UREA AND CREATININE (MMOLS/L) (MALE & FEMALE STONE FORMERS)

Calculations by Column:Including Rows 56 - 113 MALES

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
4	SE. CA	2.3437	0.1051	0.0143	4.4837	54

Calculations by Column:Including Rows 1 - 29 FEMALES

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
4	SE. CA	2.3988	0.1182	0.0241	4.9285	24

Calculations by Column:Including Rows 56 - 113 MALES

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
5	SE.URATE	0.3472	0.0888	0.0126	25.5834	50

Calculations by Column:Including Rows 1 - 29 FEMALES

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
5	SE.URATE	0.3470	0.0752	0.0157	21.6808	23

Calculations by Column:Including Rows 56 - 113 MALES

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
6	SE. UREA	5.4185	1.3991	0.1904	25.8201	54

Calculations by Column:Including Rows 1 - 29 FEMALES

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
6	SE. UREA	5.1808	1.4870	0.2916	28.7026	26

Calculations by Column:Including Rows 56 - 113 MALES

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
7	SE.CREAT	103.3750	16.1220	2.8500	15.5957	32

Calculations by Column:Including Rows 1 - 29 FEMALES

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
7	SE.CREAT	82	13.2602	3.6777	16.1710	13

24 HR URINE VOLUME (LITRES) (MALES Vs CONTROLS)
(FEMALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
8	24VOL 1	2.0189	0.6463	0.0943	47
9	24V 1 K	1.4535	0.5691	0.1273	20

F ratio = 1.2894 f1 = 46 f2 = 19 p >=0.1

Assuming Equal Variance : T = 3.3903 DF = 65 p = 0.001

Assuming Unequal Variance : T = 3.5703 DF = 41 p <0.001

Mann Whitney U Test.

Data from Column 8 and 9 including Row 1 to 134

Data set 1 24VOL 1 N = 47

Data set 2 24V 1 K N = 20

U = 239

Z = -3.1664 p = 0.002

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
10	24VOL 2	1.8033	0.8106	0.1769	21
11	24V 2 K	1.4258	0.6030	0.1231	24

F ratio = 1.8074 f1 = 20 f2 = 23 p >=0.05 & p <0.1

Assuming Equal Variance : T = 1.7867 DF = 43 p = 0.081

Assuming Unequal Variance : T = 1.7519 DF = 37 p = 0.088

Mann Whitney U Test.

Data from Column 10 and 11 including Row 1 to 134

Data set 1 24VOL 2 N = 21

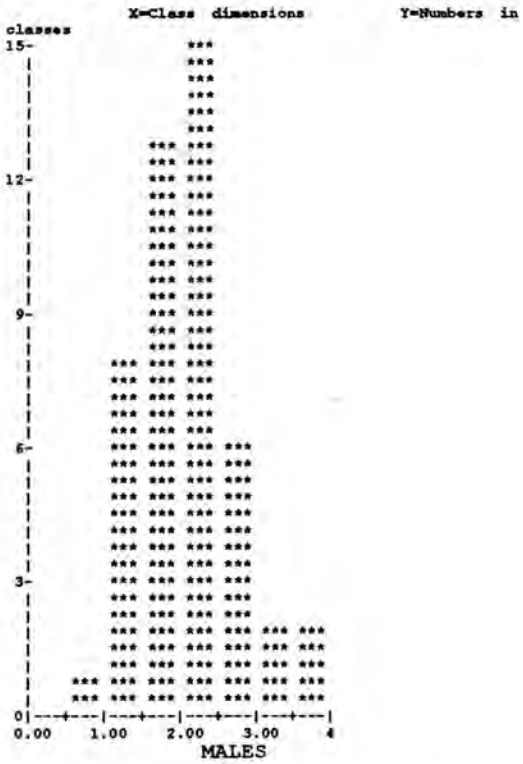
Data set 2 24V 2 K N = 24

U = 179

Z = -1.6611 p = 0.097

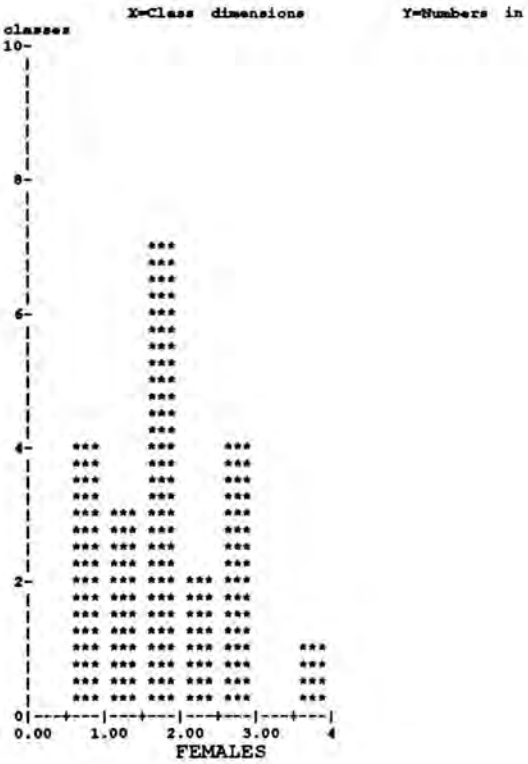
FREQUENCY DISTRIBUTION - 24 HR URINE VOLUME (L)

Data from columns 8 to 8 and rows 1 to 134



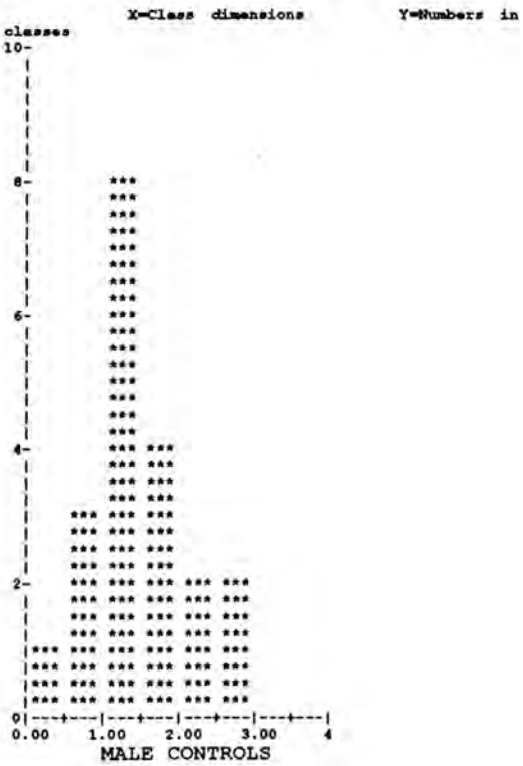
MALES

Data from columns 10 to 10 and rows 1 to 134



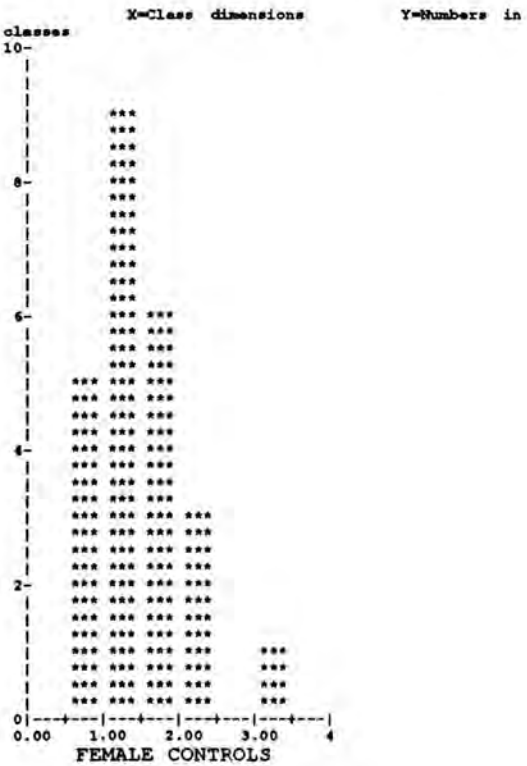
FEMALES

Data from columns 9 to 9 and rows 1 to 134



MALE CONTROLS

Data from columns 11 to 11 and rows 1 to 134



FEMALE CONTROLS

24 HR URINE CREATININE (MMOLS/24 HRS) (MALES Vs CONTROLS)
(FEMALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
12	24CR 1	13.5587	4.4368	0.6472	47
13	24CR 1 K	11.6315	3.6376	0.8134	20

F ratio = 1.4877 f1 = 46 f2 = 19 p >=0.1

Assuming Equal Variance : T = 1.7110 DF = 65 p = 0.092

Assuming Unequal Variance : T = 1.8541 DF = 43 p = 0.071

Mann Whitney U Test.

Data from Column 12 and 13 including Row 1 to 134

Data set 1 24CR 1 N = 47

Data set 2 24CR 1 K N = 20

U = 363

Z = -1.4664 p = 0.142

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
14	24CR 2	9.1919	2.8556	0.6231	21
15	24CR 2 K	9.4908	1.9413	0.3963	24

F ratio = 2.1637 f1 = 20 f2 = 23 p >=0.01 & p <0.05

Assuming Equal Variance : T = 0.4151 DF = 43 p = 0.680

Assuming Unequal Variance : T = 0.4048 DF = 35 p = 0.688

Mann Whitney U Test.

Data from Column 14 and 15 including Row 1 to 134

Data set 1 24CR 2 N = 21

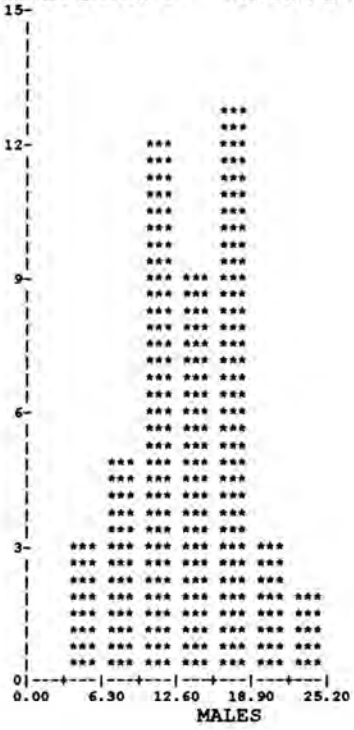
Data set 2 24CR 2 K N = 24

U = 228

Z = -0.5461 p = 0.584

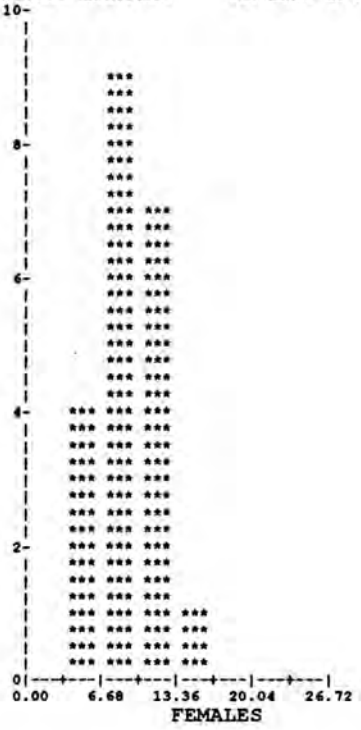
FREQUENCY DISTRIBUTION - 24 HR CREATININE (MMOLS)

Data from columns 12 to 12 and rows 1 to 134



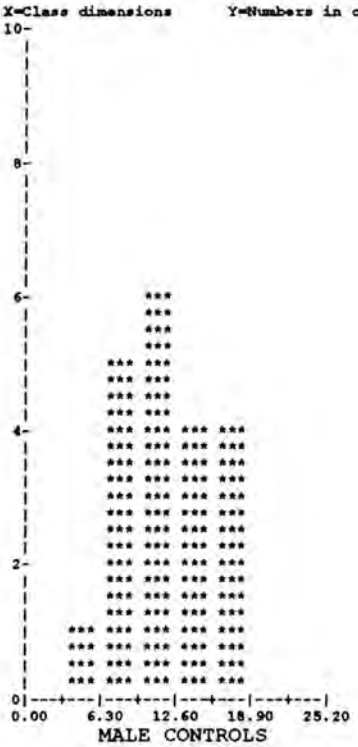
MALES

Data from columns 14 to 14 and rows 1 to 134



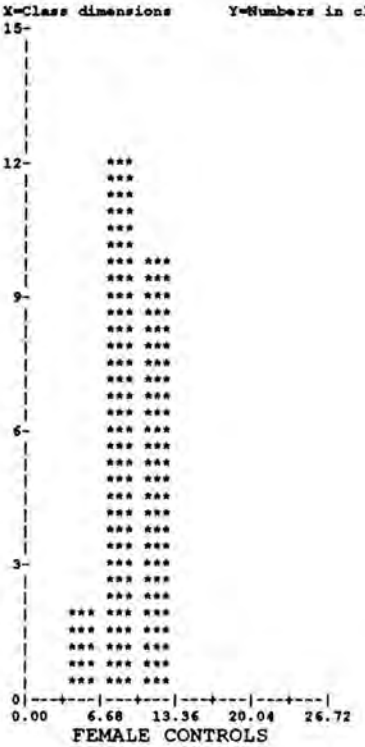
FEMALES

Data from columns 13 to 13 and rows 1 to 134



MALE CONTROLS

Data from columns 15 to 15 and rows 1 to 134



FEMALE CONTROLS

RELATIONSHIP BETWEEN 24 HR URINE CALCIUM AND CALCIFICATION ON RENAL BIOPSY

(MALES)
(FEMALES)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
1	CA+ 1 CA	7.86	2.75	0.59	22
5	CA- 1 CA	8.14	4.67	1.41	11

F ratio = 2.8799 f1 = 10 f2 = 21 p >=0.01 & p <0.05

Assuming Equal Variance : T = 0.2144 DF = 31 p = 0.832

Assuming Unequal Variance : T = 0.1811 DF = 14 p = 0.859

Mann Whitney U Test.

Data from Column 1 and 5 including Row 1 to 134

Data set 1 CA+ 1 CA N = 22

Data set 2 CA- 1 CA N = 11

U = 8

Z = -0.1146 p = 0.906

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
9	CA+ 2 CA	5.46	2.48	0.88	8
13	CA- 2 CA	6.95	3.63	1.48	6

F ratio = 2.1451 f1 = 5 f2 = 7 p >=0.1

Assuming Equal Variance : T = 0.9142 DF = 12 p = 0.379

Assuming Unequal Variance : T = 0.8638 DF = 8 p = 0.413

Mann Whitney U Test.

Data from Column 9 and 13 including Row 1 to 134

Data set 1 CA+ 2 CA N = 8

Data set 2 CA- 2 CA N = 6

U = 16

RELATIONSHIP BETWEEN 24 HR URINE URATE AND CALCIFICATION ON RENAL BIOPSY

(MALES)
(FEMALES)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
2	CA+ 1 UR	4.11	1.15	0.24	22
6	CA- 1 UR	4.16	1.79	0.57	10

F ratio = 2.4371 f1 = 9 f2 = 21 p >=0.01 & p <0.05

Assuming Equal Variance : T = 0.0955 DF = 30 p = 0.925

Assuming Unequal Variance : T = 0.0811 DF = 12 p = 0.937

Mann Whitney U Test.

Data from Column 2 and 6 including Row 1 to 134

Data set 1 CA+ 1 UR N = 22

Data set 2 CA- 1 UR N = 10

U = 101

Z = -0.3661 p = 0.713

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
10	CA+ 2 UR	3.31	0.72	0.25	8
14	CA- 2 UR	3.28	0.87	0.43	4

F ratio = 1.4613 f1 = 3 f2 = 7 p >=0.1

Assuming Equal Variance : T = 0.0802 DF = 10 p = 0.938

Assuming Unequal Variance : T = 0.0748 DF = 5 p = 0.943

Mann Whitney U Test.

Data from Column 10 and 14 including Row 1 to 134

Data set 1 CA+ 2 UR N = 8

Data set 2 CA- 2 UR N = 4

U = 16

RELATIONSHIP BETWEEN 24 HR URINE OXALATE AND CALCIFICATION ON RENAL BIOPSY

(MALES)
(FEMALES)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
4	CA+ 1 OX	0.20	0.09	0.02	21
8	CA- 1 OX	0.19	0.08	0.03	9

F ratio = 1.4602 f1 = 20 f2 = 8 p >=0.1

Assuming Equal Variance : T = 0.1775 DF = 28 p = 0.860

Assuming Unequal Variance : T = 0.1917 DF = 18 p = 0.850

Mann Whitney U Test.

Data from Column 4 and 8 including Row 1 to 134

Data set 1 CA+ 1 OX N = 21

Data set 2 CA- 1 OX N = 9

U = 93

Z = -0.0681 p = 0.943

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
12	CA+ 2 OX	0.14	0.05	0.02	5
16	CA- 2 OX	0.18	0.05	0.02	5

F ratio = 1.0560 f1 = 4 f2 = 4 p >=0.1

Assuming Equal Variance : T = 1.2050 DF = 8 p = 0.263

Assuming Unequal Variance : T = 1.2050 DF = 8 p = 0.263

Mann Whitney U Test.

Data from Column 12 and 16 including Row 1 to 134

Data set 1 CA+ 2 OX N = 5

Data set 2 CA- 2 OX N = 5

U = 8

RELATIONSHIP BETWEEN 24 HR URINE CITRATE AND CALCIFICATION ON RENAL BIOPSY

(MALES)
(FEMALES)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
3	CA+ 1 CI	2.37	1.39	0.30	21
7	CA- 1 CI	1.25	0.92	0.31	9

F ratio = 2.2630 f1 = 20 f2 = 8 p >=0.1

Assuming Equal Variance : T = 2.2217 DF = 28 p = 0.035

Assuming Unequal Variance : T = 2.6094 DF = 23 p = 0.016

Mann Whitney U Test.

Data from Column 3 and 7 including Row 1 to 134

Data set 1 CA+ 1 CI N = 21

Data set 2 CA- 1 CI N = 9

U = 40

Z = -2.4687 p = 0.014

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
11	CA+ 2 CI	2.05	1.83	0.82	5
15	CA- 2 CI	4.15	2.59	1.16	5

F ratio = 2.0057 f1 = 4 f2 = 4 p >=0.1

Assuming Equal Variance : T = 1.4794 DF = 8 p = 0.177

Assuming Unequal Variance : T = 1.4794 DF = 7 p = 0.183

Mann Whitney U Test.

Data from Column 11 and 15 including Row 1 to 134

Data set 1 CA+ 2 CI N = 5

Data set 2 CA- 2 CI N = 5

U = 5

24 HR URINE CALCIUM (MMOLS)
CALCIUM/CREATININE RATIO

(MALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
16	24CA 1	8.1312	3.3038	0.4376	57
17	24CA 1 K	4.7840	2.8271	0.6322	20

F ratio = 1.3656 f1 = 56 f2 = 19 p >=0.1

Assuming Equal Variance : T = 4.0377 DF = 75 p <0.001

Assuming Unequal Variance : T = 4.3536 DF = 39 p <0.001

Mann Whitney U Test.

Data from Column 16 and 17 including Row 1 to 134

Data set 1 24CA 1 N = 57

Data set 2 24CA 1 K N = 20

U = 247

Z = -3.7527 p = < 0.001

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
32	CA 1/CR	0.6977	0.4582	0.0676	46
34	CA 1K/CR	0.4081	0.1781	0.0398	20

F ratio = 6.6187 f1 = 45 f2 = 19 p <0.01

Assuming Equal Variance : T = 2.7285 DF = 64 p = 0.008

Assuming Unequal Variance : T = 3.6929 DF = 64 p <0.001

Mann Whitney U Test.

Data from Column 32 and 34 including Row 1 to 134

Data set 1 CA 1/CR N = 46

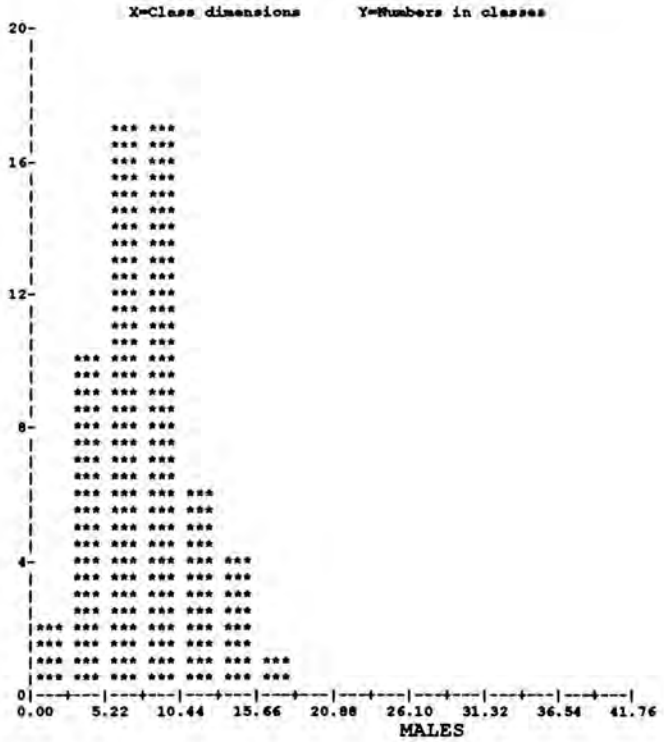
Data set 2 CA 1K/CR N = 20

U = 216

Z = -3.4045 p = < 0.001

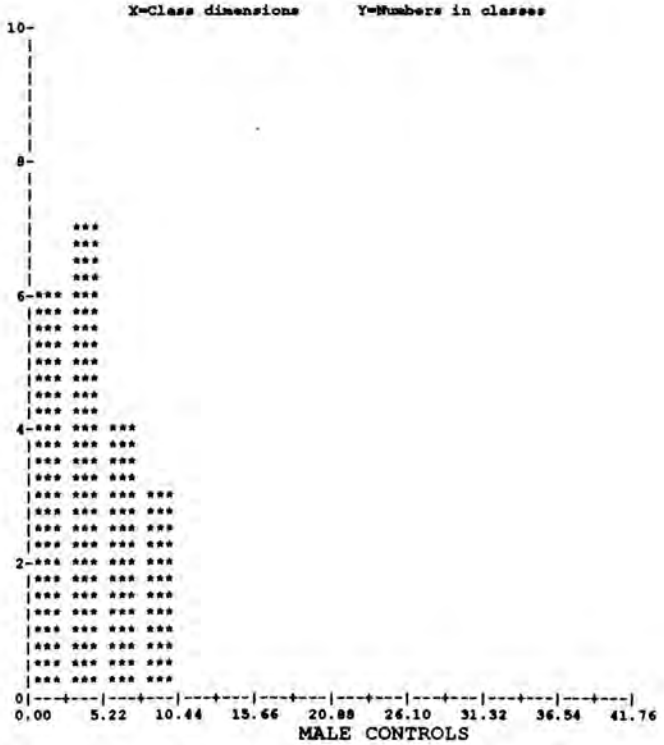
FREQUENCY DISTRIBUTION - 24 HR URINE CALCIUM (MMOLS)
(Control Mean + 2 S.D. = 10.44)

Data from columns 16 to 16 and rows 1 to 134



MALES

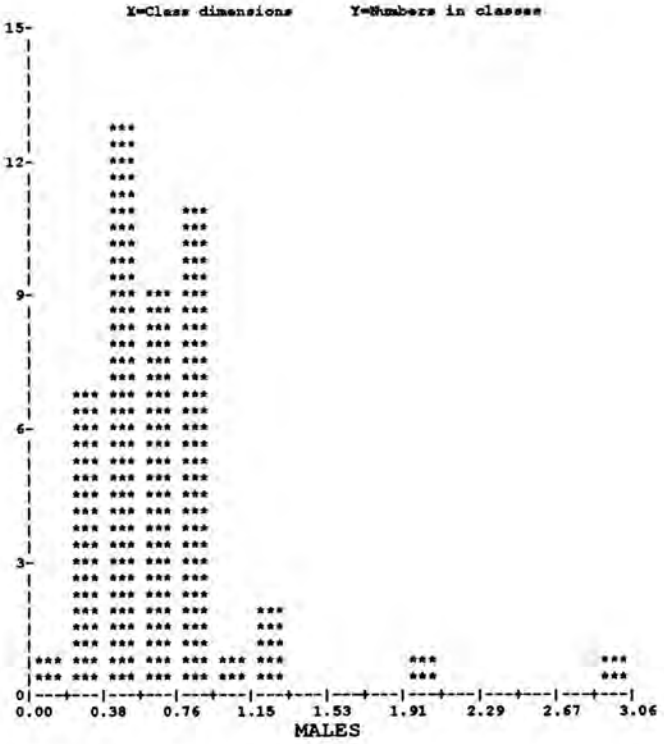
Data from columns 17 to 17 and rows 1 to 134



MALE CONTROLS

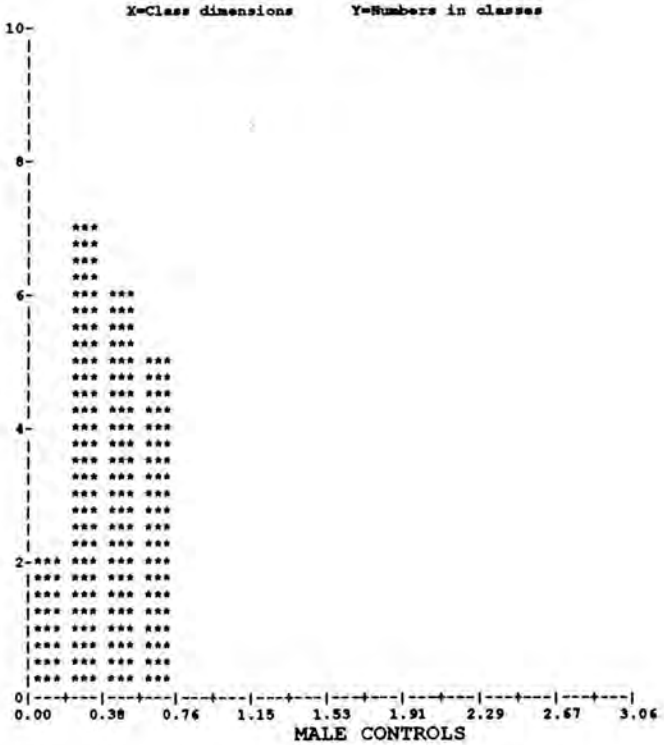
FREQUENCY DISTRIBUTION - URINE CALCIUM/CREATININE RATIO
(Control Mean + 2 S.D. = 0.765)

Data from columns 32 to 32 and rows 1 to 134



MALES

Data from columns 34 to 34 and rows 1 to 134



MALE CONTROLS

24 HR URINE CALCIUM (MMOLS)
CALCIUM/CREATININE RATIO

(FEMALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
18	24CA 2	5.9161	3.4215	0.6466	28
19	24CA 2 K	2.8554	1.5603	0.3185	24

F ratio = 4.8084 f1 = 27 f2 = 23 p <0.01

Assuming Equal Variance : T = 4.0333 DF = 50 p <0.001

Assuming Unequal Variance : T = 4.2462 DF = 39 p <0.001

Mann Whitney U Test.

Data from Column 18 and 19 including Row 1 to 134

Data set 1 24CA 2 N = 28

Data set 2 24CA 2 K N = 24

U = 139

Z = -3.6167 p = < 0.001

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
33	CA 2/CR	0.5791	0.3162	0.0707	20
35	CA 2K/CR	0.3224	0.2120	0.0433	24

F ratio = 2.2254 f1 = 19 f2 = 23 p >=0.01 & p <0.05

Assuming Equal Variance : T = 3.2089 DF = 42 p = 0.003

Assuming Unequal Variance : T = 3.0973 DF = 32 p = 0.004

Mann Whitney U Test.

Data from Column 33 and 35 including Row 1 to 134

Data set 1 CA 2/CR N = 20

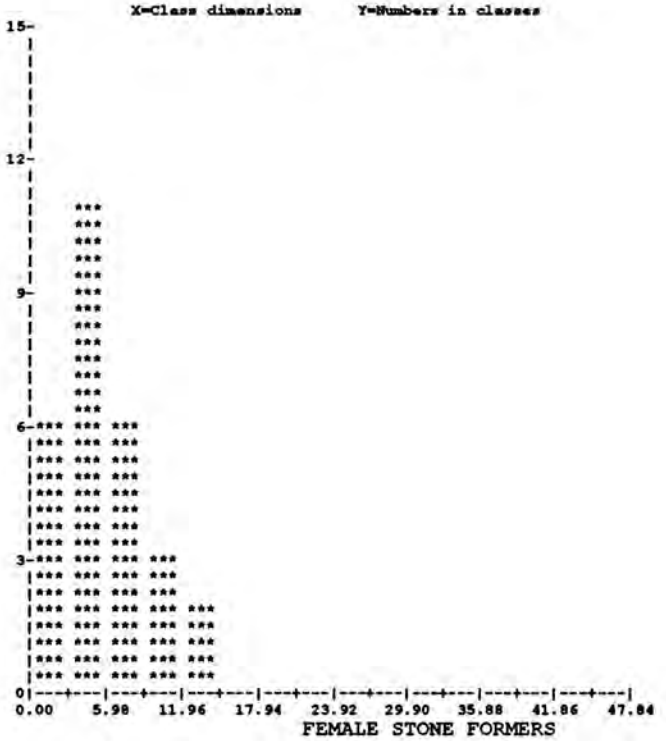
Data set 2 CA 2K/CR N = 24

U = 119

Z = -2.8520 p = 0.004

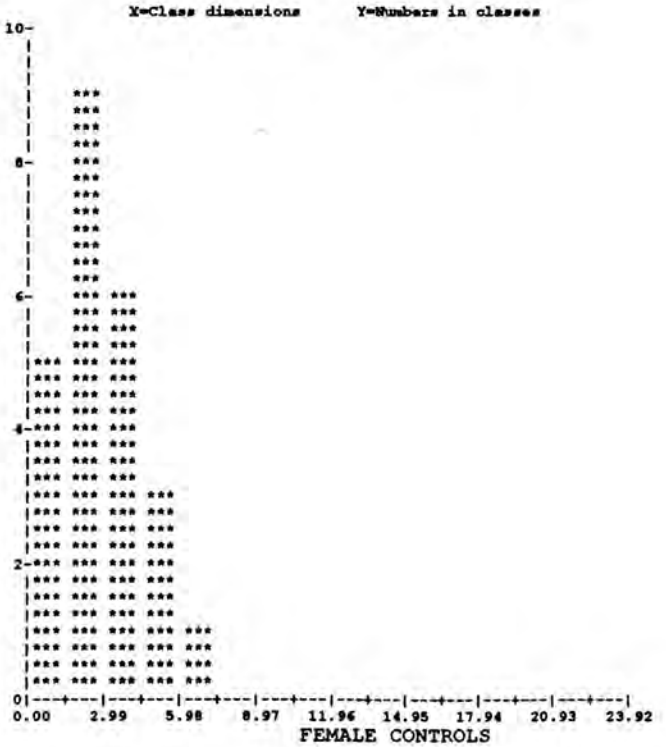
FREQUENCY DISTRIBUTION - 24 HR URINE CALCIUM
(Control Mean + 2 S.D. = 5.98)

Data from columns 18 to 18 and rows 1 to 134



FEMALE STONE FORMERS

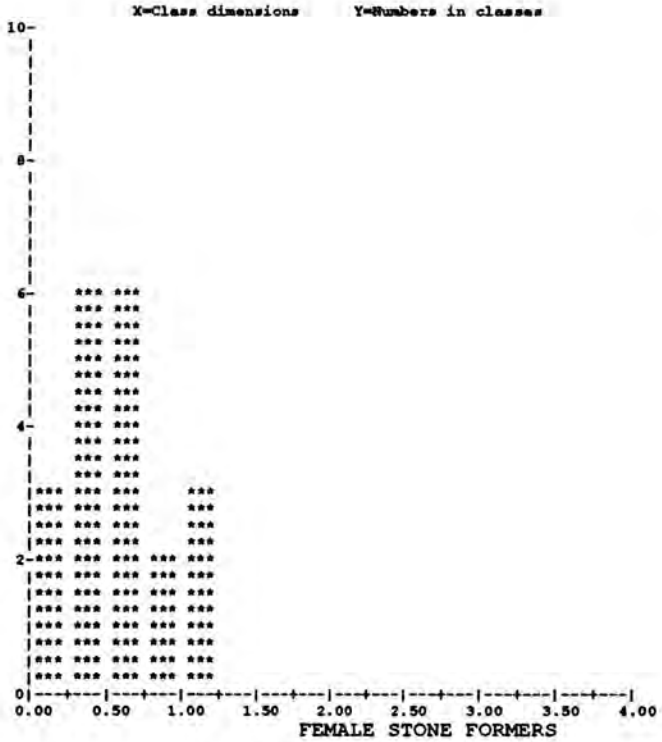
Data from columns 19 to 19 and rows 1 to 134



FEMALE CONTROLS

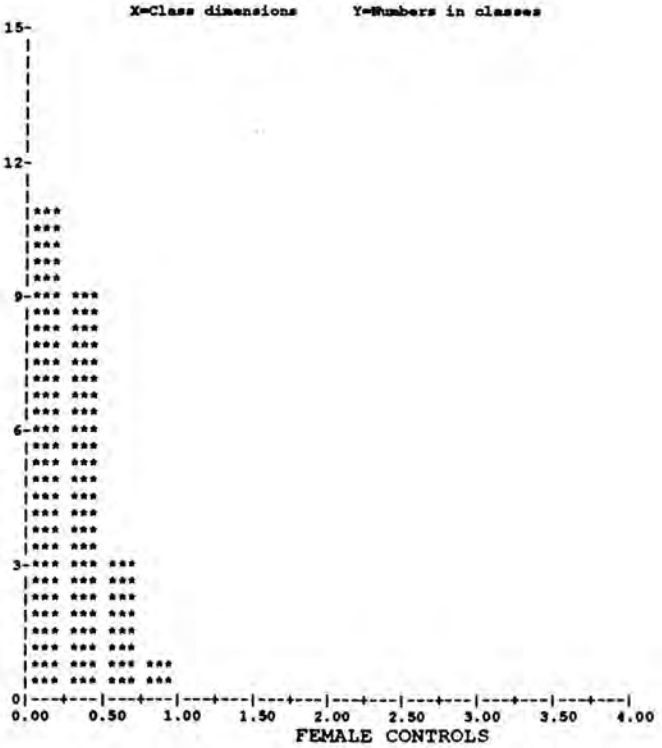
FREQUENCY DISTRIBUTION - 24 HR URINE CALCIUM/CREATININE RATIO
(Control Mean + 2 S.D. = 0.746)

Data from columns 33 to 33 and rows 1 to 134



FEMALE STONE FORMERS

Data from columns 35 to 35 and rows 1 to 134



FEMALE CONTROLS

24 HR URINE URATE (MMOLS)
URATE/CREATININE RATIO

(MALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
20	24UR 1	4.5386	1.4994	0.2004	56
21	24UR 1 K	2.7115	1.4293	0.3196	20

F ratio = 1.1004 f1 = 55 f2 = 19 p >=0.1

Assuming Equal Variance : T = 4.7336 DF = 74 p <0.001

Assuming Unequal Variance : T = 4.8435 DF = 35 p <0.001

Mann Whitney U Test.

Data from Column 20 and 21 including Row 1 to 134

Data set 1 24UR 1 N = 56

Data set 2 24UR 1 K N = 20

U = 193

z = -4.3294 p = < 0.001

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
36	UR 1/CR	0.3664	0.1751	0.0261	45
38	UR 1K/CR	0.2505	0.1389	0.0311	20

F ratio = 1.5894 f1 = 44 f2 = 19 p >=0.1

Assuming Equal Variance : T = 2.6130 DF = 63 p = 0.011

Assuming Unequal Variance : T = 2.8564 DF = 46 p = 0.006

Mann Whitney U Test.

Data from Column 36 and 38 including Row 1 to 134

Data set 1 UR 1/CR N = 45

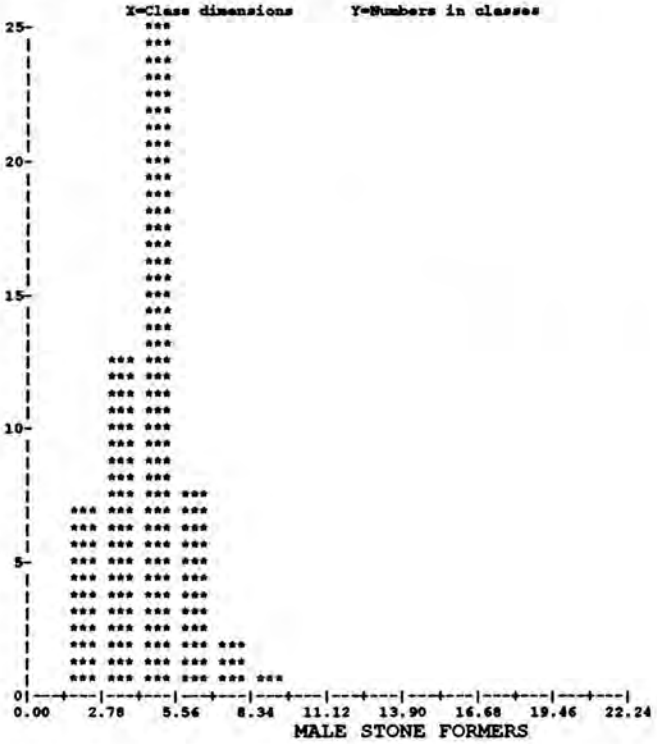
Data set 2 UR 1K/CR N = 20

U = 268

z = -2.5869 p = 0.010

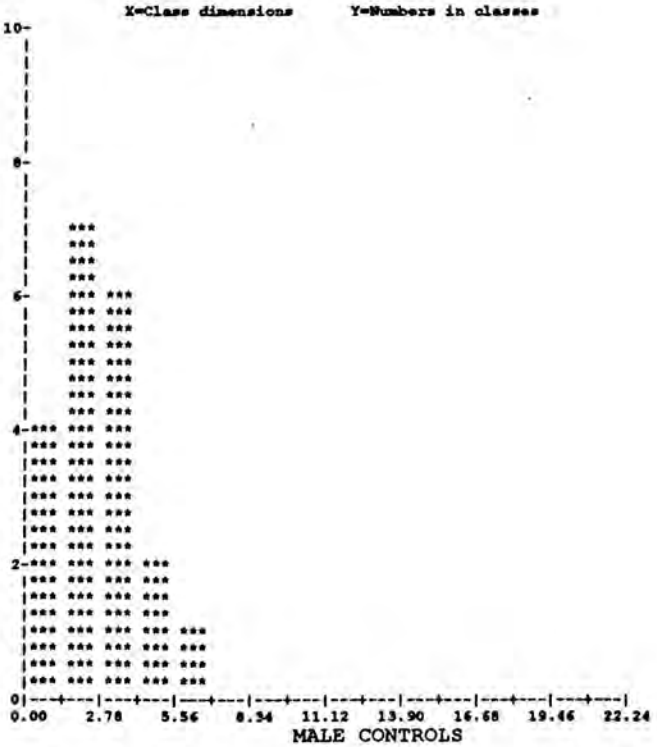
FREQUENCY DISTRIBUTION - 24 HR URINE URATE
(Control Mean + 2 S.D. = 5.56)

Data from columns 20 to 20 and rows 1 to 134



MALE STONE FORMERS

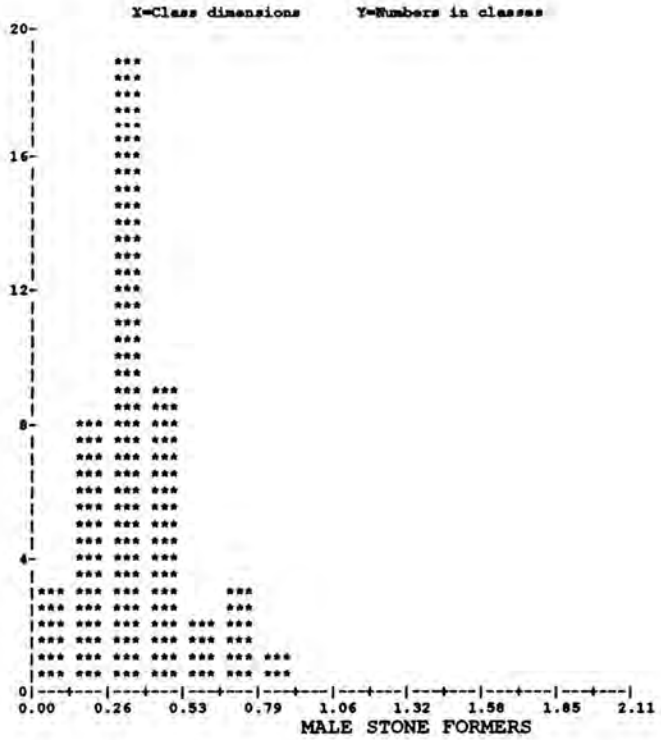
Data from columns 21 to 21 and rows 1 to 134



MALE CONTROLS

FREQUENCY DISTRIBUTION - 24 HR URINE URATE/CREATININE RATIO
(Control Mean + 2 S.D. = 0.528)

Data from columns 36 to 36 and rows 1 to 134



MALE STONE FORMERS

Data from columns 38 to 38 and rows 1 to 134

MALE CONTROLS

24 HR URINE URATE (MMOLS)
URATE/CREATININE RATIO

(FEMALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
22	24UR 2	3.4036	1.3054	0.2611	25
23	24UR 2 K	3.4279	1.4937	0.3049	24

F ratio = 1.3092 f1 = 23 f2 = 24 p >=0.1

Assuming Equal Variance : T = 0.0607 DF = 47 p = 0.952

Assuming Unequal Variance : T = 0.0606 DF = 46 p = 0.952

Mann Whitney U Test.

Data from Column 22 and 23 including Row 1 to 134

Data set 1 24UR 2 N = 25

Data set 2 24UR 2 K N = 24

U = 289

Z = -0.2200 p = 0.824

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
37	UR 2/CR	0.3935	0.1674	0.0406	17
39	UR 2K/CR	0.3540	0.1129	0.0231	24

F ratio = 2.1962 f1 = 16 f2 = 23 p >=0.01 & p <0.05

Assuming Equal Variance : T = 0.9039 DF = 39 p = 0.372

Assuming Unequal Variance : T = 0.8464 DF = 26 p = 0.405

Mann Whitney U Test.

Data from Column 37 and 39 including Row 1 to 134

Data set 1 UR 2/CR N = 17

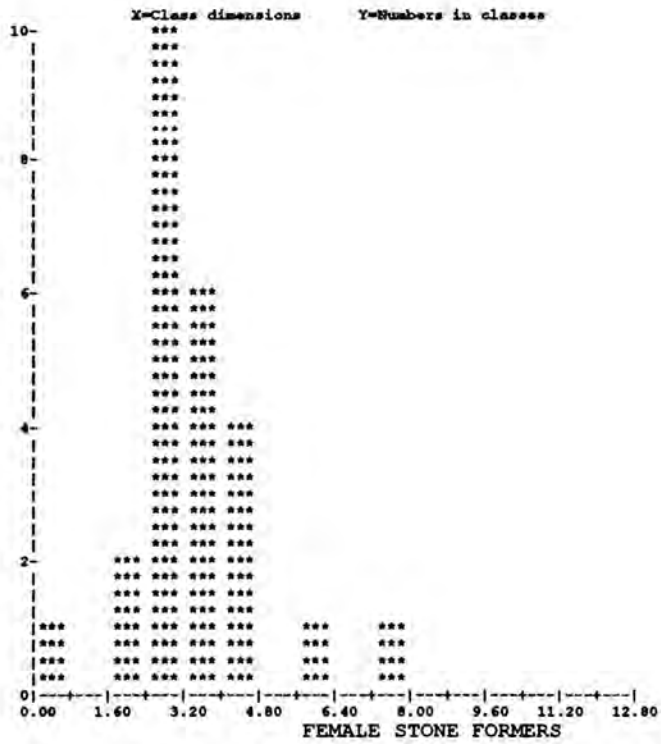
Data set 2 UR 2K/CR N = 24

U = 179

Z = -0.6616 p = 0.508

FREQUENCY DISTRIBUTION - 24 HR URINE URATE
(Control Mean + 2 S.D. = 6.40)

Data from columns 22 to 22 and rows 1 to 134



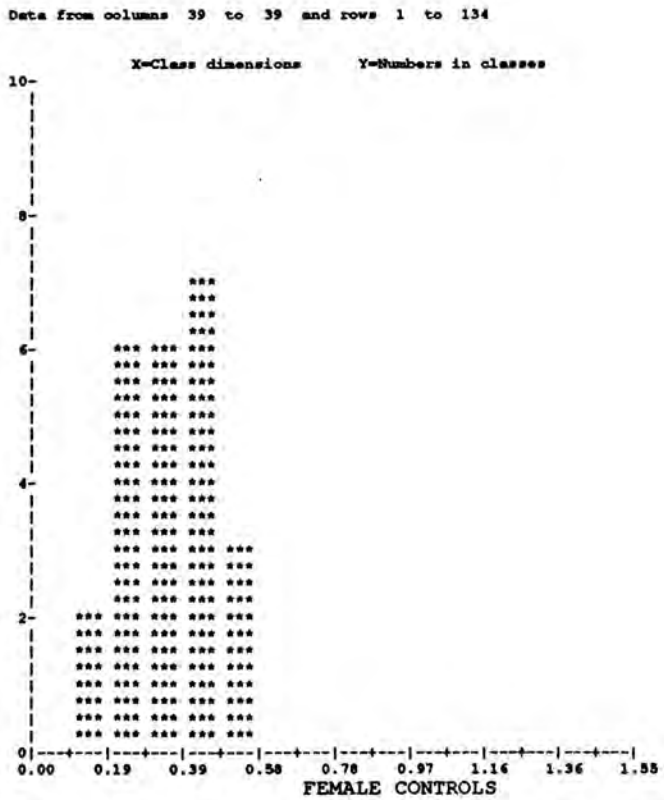
FEMALE STONE FORMERS

Data from columns 23 to 23 and rows 1 to 134

FEMALE CONTROLS

FREQUENCY DISTRIBUTION - 24 HR URINE URATE/CREATININE RATIO
(Control Mean + 2 S.D. = 0.580)

FEMALE STONE FORMERS



FEMALE CONTROLS

24 HR URINE OXALATE (MMOLS)
OXALATE/CREATININE RATIO

(MALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
24	24OX 1	0.2131	0.1170	0.0171	47
26	24OX 1 K	0.2285	0.1145	0.0256	20

F ratio = 1.0436 f1 = 46 f2 = 19 p >=0.1

Assuming Equal Variance : T = 0.4973 DF = 65 p = 0.621

Assuming Unequal Variance : T = 0.5017 DF = 37 p = 0.619

Mann Whitney U Test.

Data from Column 24 and 26 including Row 1 to 134

Data set 1 24OX 1 N = 47

Data set 2 24OX 1 K N = 20

U = 397

Z = -1.0003 p = 0.317

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
40	OX 1/CR	0.0168	0.0085	0.0012	47
42	OX 1K/CR	0.0198	0.0080	0.0018	20

F ratio = 1.1361 f1 = 46 f2 = 19 p >=0.1

Assuming Equal Variance : T = 1.3431 DF = 65 p = 0.184

Assuming Unequal Variance : T = 1.3786 DF = 38 p = 0.176

Mann Whitney U Test.

Data from Column 40 and 42 including Row 1 to 134

Data set 1 OX 1/CR N = 47

Data set 2 OX 1K/CR N = 20

U = 358

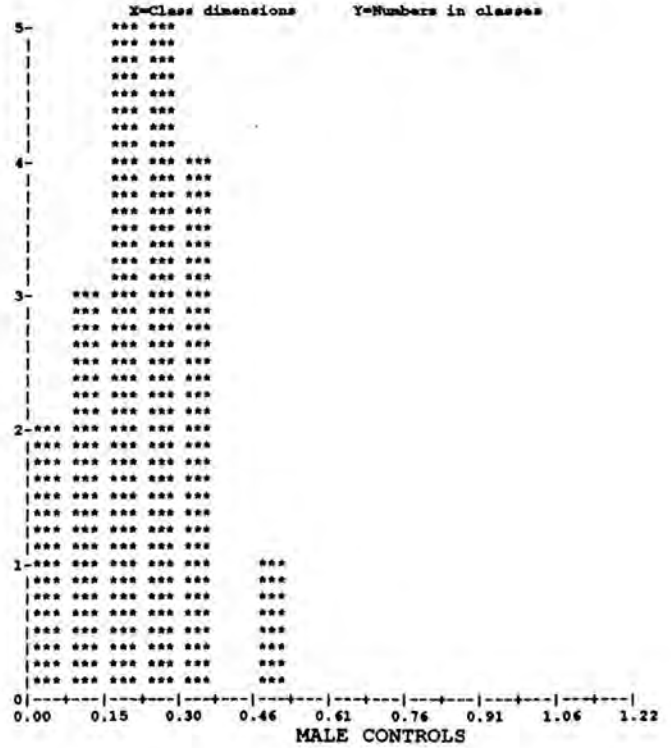
Z = -1.5346 p = 0.125

FREQUENCY DISTRIBUTION - 24 HR URINE OXALATE
(Control Mean + 2 S.D. = 0.456)

Data from columns 24 to 24 and rows 1 to 134

MALE STONE FORMERS

Data from columns 26 to 26 and rows 1 to 134



MALE CONTROLS

FREQUENCY DISTRIBUTION - 24 HR URINE OXALATE/CREATININE RATIO
(Control Mean + 2 S.D. = 0.04)

24 HR URINE OXALATE (MMOLS)
OXALATE/CREATININE RATIO

(FEMALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
25	24OX 2	0.1836	0.0837	0.0183	21
27	24OX 2 K	0.2303	0.1335	0.0273	24

F ratio = 2.5424 f1 = 23 f2 = 20 p >=0.01 & p <0.05

Assuming Equal Variance : T = 1.3830 DF = 43 p = 0.174

Assuming Unequal Variance : T = 1.4247 DF = 39 p = 0.162

Mann Whitney U Test.

Data from Column 25 and 27 including Row 1 to 134

Data set 1 24OX 2 N = 21

Data set 2 24OX 2 K N = 24

U = 216

Z = -0.8191 p = 0.412

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
41	OX 2/CR	0.0201	0.0081	0.0018	21
43	OX 2K/CR	0.0240	0.0118	0.0024	24

F ratio = 2.0941 f1 = 23 f2 = 20 p >=0.01 & p <0.05

Assuming Equal Variance : T = 1.2706 DF = 43 p = 0.211

Assuming Unequal Variance : T = 1.3016 DF = 41 p = 0.200

Mann Whitney U Test.

Data from Column 41 and 43 including Row 1 to 134

Data set 1 OX 2/CR N = 21

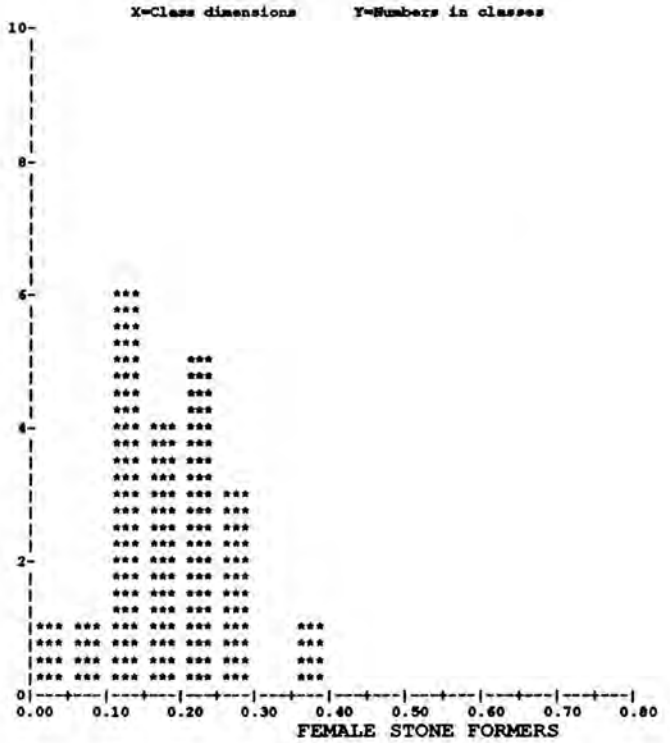
Data set 2 OX 2K/CR N = 24

U = 206

Z = -1.0465 p = 0.295

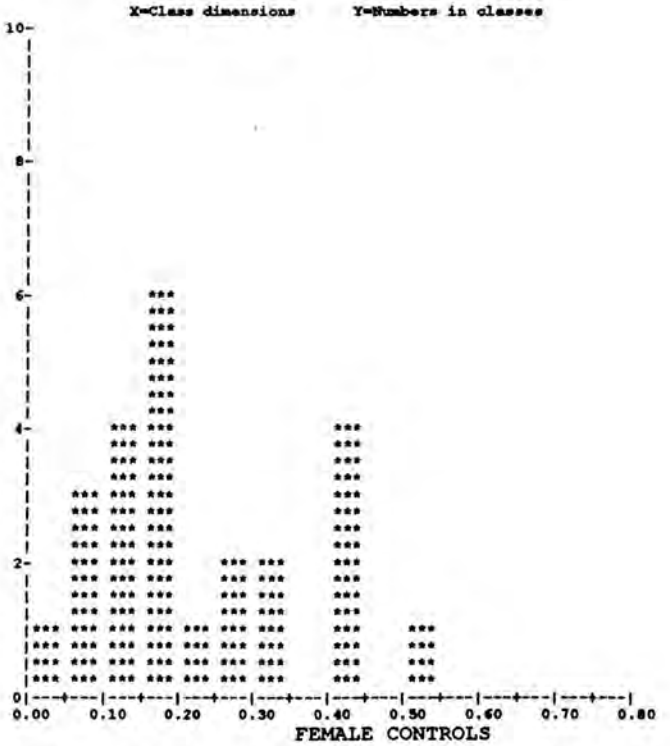
FREQUENCY DISTRIBUTION - 24 HR URINE OXALATE
(Control Mean + 2 S.D. = 0.497)

Data from columns 25 to 25 and rows 1 to 134



FEMALE STONE FORMERS

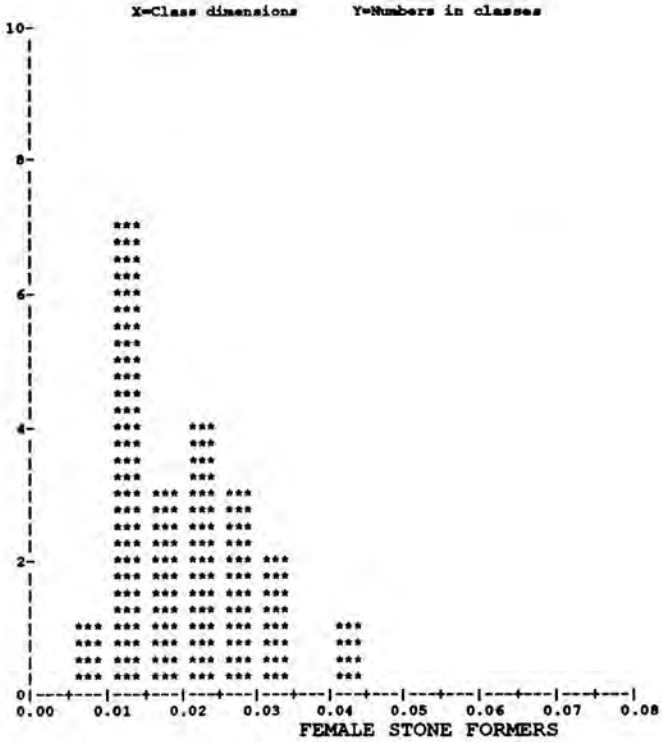
Data from columns 27 to 27 and rows 1 to 134



FEMALE CONTROLS

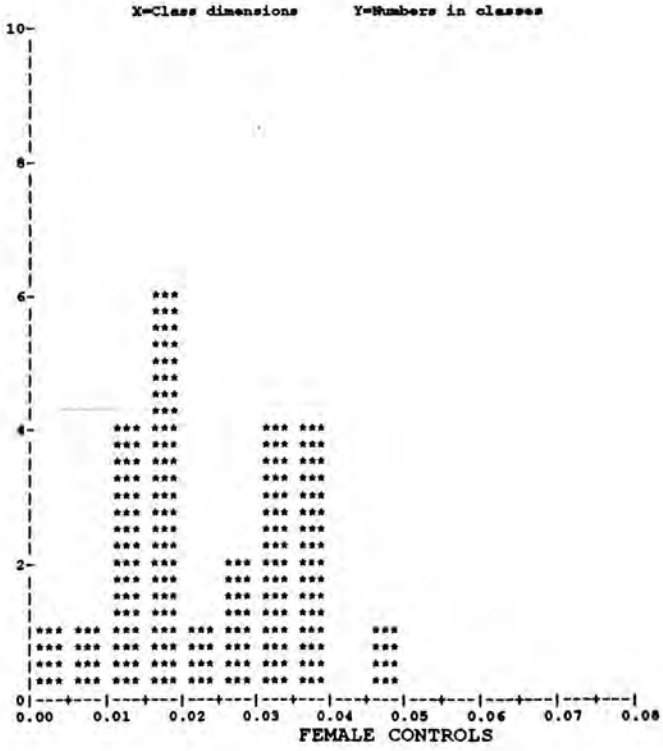
FREQUENCY DISTRIBUTION - 24 HR URINE OXALATE/CREATININE RATIO
(Control Mean + 2 S.D. = 0.048)

Data from columns 41 to 41 and rows 1 to 134



FEMALE STONE FORMERS

Data from columns 43 to 43 and rows 1 to 134



FEMALE CONTROLS

24 HR URINE CITRATE (MMOLS)
CITRATE/CREATININE RATIO

(MALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
28	24CIT 1	2.3837	1.3317	0.1942	47
30	24CIT 1K	2.4589	1.6627	0.3718	20

F ratio = 1.5588 f1 = 19 f2 = 46 p >=0.05 & p <0.1

Assuming Equal Variance : T = 0.1960 DF = 65 p = 0.845

Assuming Unequal Variance : T = 0.1792 DF = 30 p = 0.859

Mann Whitney U Test.

Data from Column 28 and 30 including Row 1 to 134

Data set 1 24CIT 1 N = 47

Data set 2 24CIT 1K N = 20

U = 464

Z = -0.0822 p = 0.932

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
44	CT/CR 1	0.1921	0.1307	0.0191	47
46	CT/CR 1K	0.2062	0.1226	0.0274	20

F ratio = 1.1362 f1 = 46 f2 = 19 p >=0.1

Assuming Equal Variance : T = 0.4109 DF = 65 p = 0.683

Assuming Unequal Variance : T = 0.4217 DF = 38 p = 0.676

Mann Whitney U Test.

Data from Column 44 and 46 including Row 1 to 134

Data set 1 CT/CR 1 N = 47

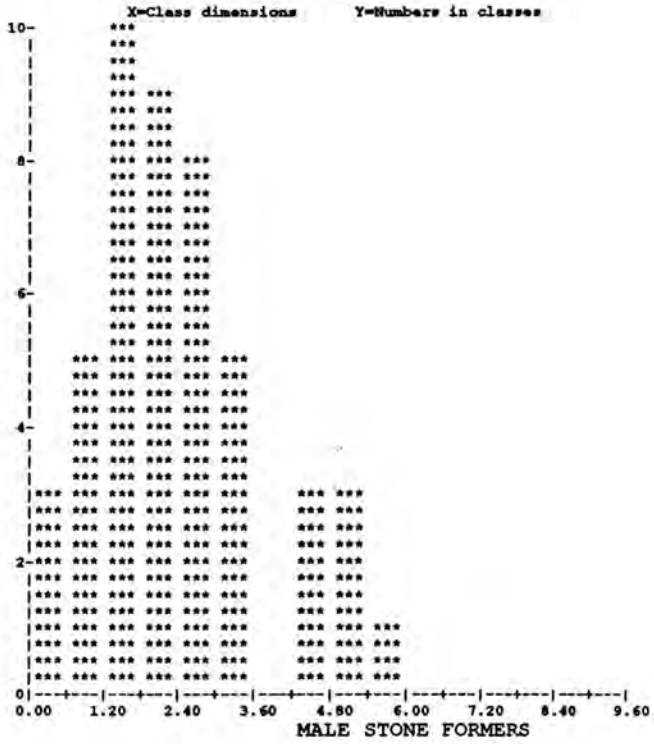
Data set 2 CT/CR 1K N = 20

U = 422

Z = -0.6577 p = 0.510

FREQUENCY DISTRIBUTION - 24 HR URINE CITRATE

Data from columns 28 to 28 and rows 1 to 134



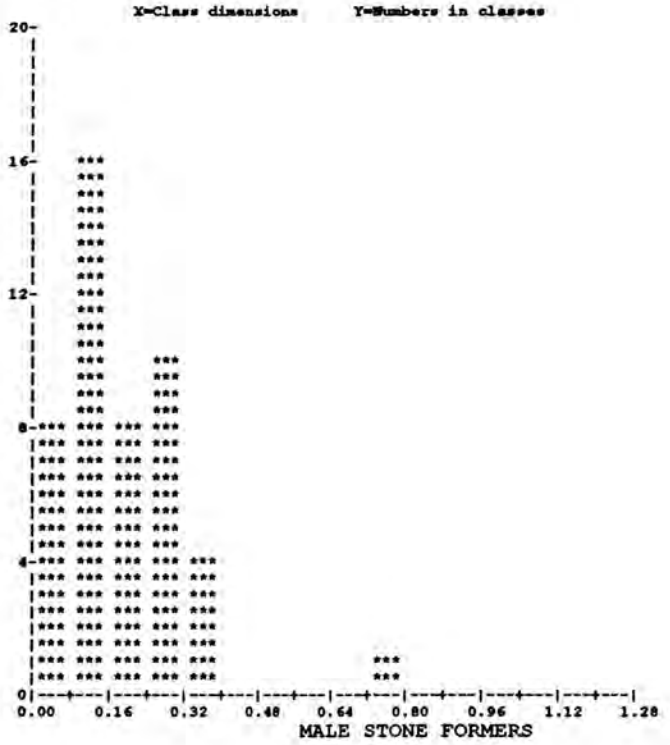
MALE STONE FORMERS

Data from columns 30 to 30 and rows 1 to 134

MALE CONTROLS

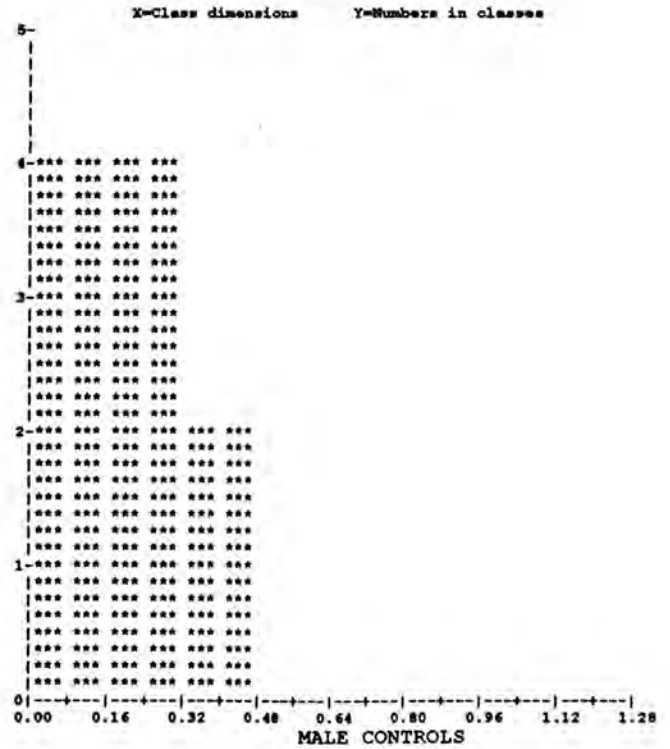
FREQUENCY DISTRIBUTION - 24 HR URINE CITRATE/CREATININE RATIO

Data from columns 44 to 44 and rows 1 to 134



MALE STONE FORMERS

Data from columns 46 to 46 and rows 1 to 134



MALE CONTROLS

24 HR URINE CITRATE (MMOLS)
CITRATE/CREATININE RATIO

(FEMALES Vs CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
29	24CIT 2	2.5648	2.0541	0.4482	21
31	24CIT 2K	4.0785	2.6890	0.5489	24

F ratio = 1.7138 f1 = 23 f2 = 20 p >=0.05 & p <0.1

Assuming Equal Variance : T = 2.0980 DF = 43 p = 0.042

Assuming Unequal Variance : T = 2.1360 DF = 42 p = 0.039

Mann Whitney U Test.

Data from Column 29 and 31 including Row 1 to 134

Data set 1 24CIT 2 N = 21

Data set 2 24CIT 2K N = 24

U = 168

Z = -1.9112 p = 0.056

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
45	CT/CR 2	0.2596	0.1875	0.0409	21
47	CT/CR 2K	0.4403	0.2781	0.0568	24

F ratio = 2.2011 f1 = 23 f2 = 20 p >=0.01 & p <0.05

Assuming Equal Variance : T = 2.5164 DF = 43 p = 0.016

Assuming Unequal Variance : T = 2.5816 DF = 41 p = 0.014

Mann Whitney U Test.

Data from Column 45 and 47 including Row 1 to 134

Data set 1 CT/CR 2 N = 21

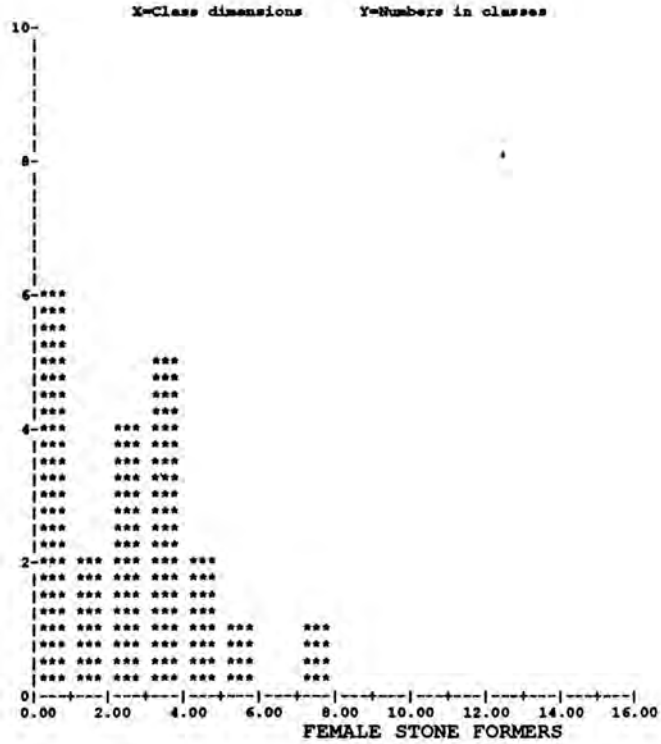
Data set 2 CT/CR 2K N = 24

U = 155

Z = -2.2068 p = 0.027

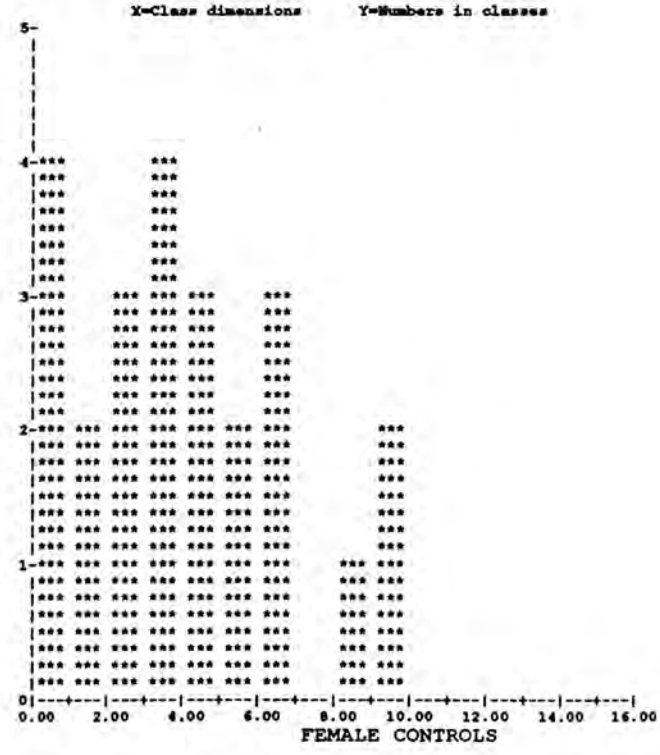
FREQUENCY DISTRIBUTION - 24 HR URINE CITRATE

Data from columns 29 to 29 and rows 1 to 134



FEMALE STONE FORMERS

Data from columns 31 to 31 and rows 1 to 134



FEMALE CONTROLS

FREQUENCY DISTRIBUTION - 24 HR URINE CITRATE/CREATININE RATIO

Data from columns 45 to 45 and rows 1 to 134

FEMALE STONE FORMERS

Data from columns 47 to 47 and rows 1 to 134

FEMALE CONTROLS

24 HR URINE CITRATE/CREATININE RATIO - (MALE CONTROLS Vs FEMALE CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
46	CT/CR 1K	0.2062	0.1226	0.0274	20
47	CT/CR 2K	0.4403	0.2781	0.0568	24

F ratio = 5.1444 f1 = 23 f2 = 19 p <0.01

Assuming Equal Variance : T = 3.4861 DF = 42 p = 0.001

Assuming Unequal Variance : T = 3.7119 DF = 33 p <0.001

Mann Whitney U Test.

Data from Column 46 and 47 including Row 1 to 134

Data set 1 CT/CR 1K N = 20

Data set 2 CT/CR 2K N = 24

U = 112

Z = -3.0171 p = 0.003

24 HR URINE CITRATE/CREATININE RATIO - (After deletion of all values < 0.16)
(FEMALE STONE FORMERS & CONTROLS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
45	CT/CR 2	0.3800	0.1272	0.0353	13
47	CT/CR 2K	0.5202	0.2305	0.0515	20

F ratio = 3.2833 f1 = 19 f2 = 12 p >=0.01 & p <0.05

Assuming Equal Variance : T = 1.9980 DF = 31 p = 0.055

Assuming Unequal Variance : T = 2.2455 DF = 30 p = 0.032

Mann Whitney U Test.

Data from Column 45 and 47 including Row 1 to 134

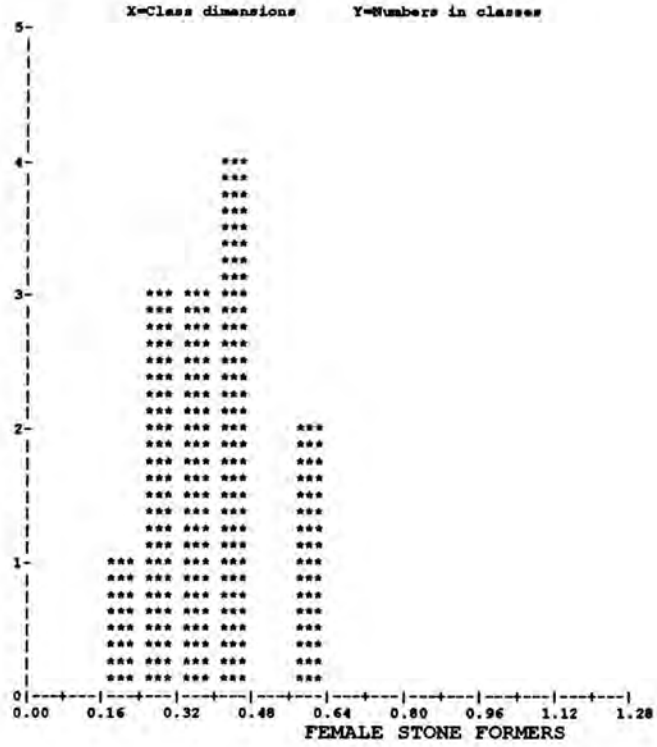
Data set 1 CT/CR 2 N = 13

Data set 2 CT/CR 2K N = 20

U = 81

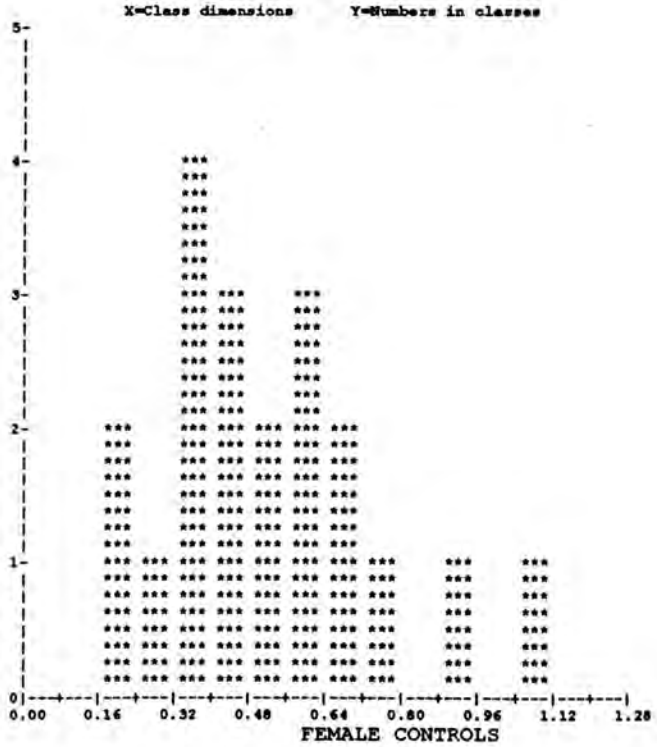
FREQUENCY DISTRIBUTION - 24 HR URINE CITRATE/CREATININE RATIO
(After deletion of values <0.16)

Data from columns 45 to 45 and rows 1 to 134



FEMALE STONE FORMERS

Data from columns 47 to 47 and rows 1 to 134



FEMALE CONTROLS

24 HR URINE CITRATE (MMOLS) - After deletion of patients whose Calcium or Urate or Oxalate was > (control mean + 2 S.D.)

(MALES)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
28	24CIT 1	2.3745	1.3422	0.2451	30
30	24CIT 1K	2.2810	1.5866	0.3740	18

F ratio = 1.3974 f1 = 17 f2 = 29 p >=0.1

Assuming Equal Variance : T = 0.2182 DF = 46 p = 0.828

Assuming Unequal Variance : T = 0.2091 DF = 31 p = 0.836

Mann Whitney U Test.

Data from Column 28 and 30 including Row 1 to 134

Data set 1 24CIT 1 N = 30

Data set 2 24CIT 1K N = 18

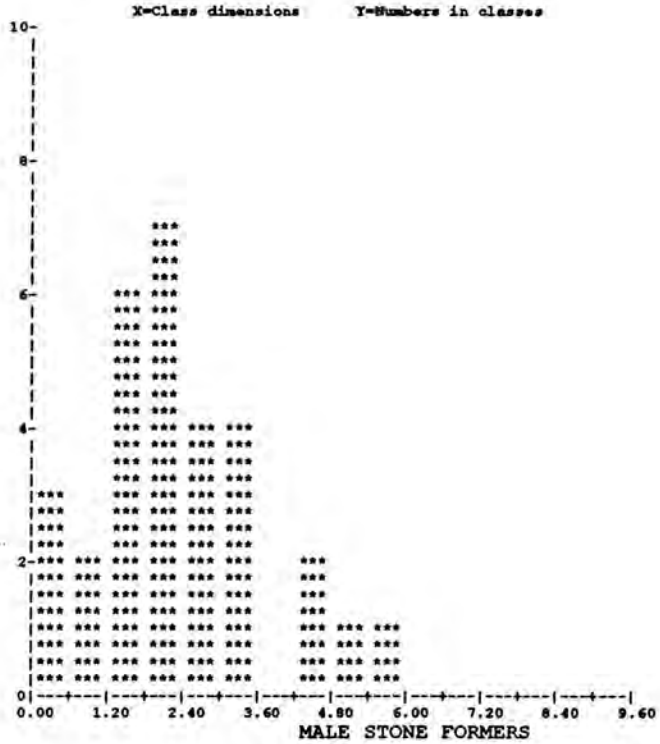
U = 250

Z = -0.4259 p = 0.669

FREQUENCY DISTRIBUTION

24 HR URINE CITRATE (MMOLS) - After deletion of patients whose Calcium or Urate or Oxalate was > (control mean + 2 S.D.)

Data from columns 28 to 28 and rows 1 to 134



MALE STONE FORMERS

Data from columns 30 to 30 and rows 1 to 134

MALE CONTROLS

24 HR URINE CITRATE/CREATININE RATIOS - (MALE STONE FORMERS and CONTROLS)
After deletion of patients whose Calcium or Urate
or Oxalate was > (control mean + 2 S.D.)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
44	CT/CR 1	0.1774	0.1496	0.0283	28
46	CT/CR 1K	0.1946	0.1140	0.0262	19

F ratio = 1.7216 f1 = 27 f2 = 18 p >=0.1

Assuming Equal Variance : T = 0.4221 DF = 45 p = 0.675

Assuming Unequal Variance : T = 0.4446 DF = 44 p = 0.659

Mann Whitney U Test.

Data from Column 44 and 46 including Row 1 to 134

Data set 1 CT/CR 1 N = 28

Data set 2 CT/CR 1K N = 19

U = 220

Z = -0.9972 p = 0.318

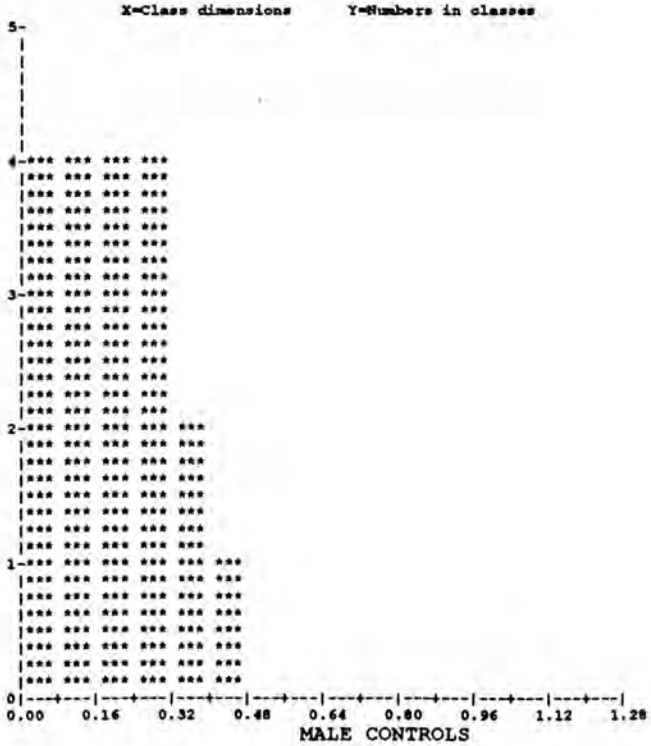
FREQUENCY DISTRIBUTION

24 HR URINE CITRATE/CREATININE RATIO -
After deletion of patients whose Calcium or Urate
or Oxalate was > (control mean + 2 S.D.)

Data from columns 44 to 44 and rows 1 to 134

MALE STONE FORMERS

Data from columns 46 to 46 and rows 1 to 134



MALE CONTROLS

24 HR URINE CITRATE (MMOLS) - (FEMALE STONE FORMERS and CONTROLS)
After deletion of patients whose Calcium or Urate
or Oxalate was > (control mean + 2 S.D.)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
29	24CIT 2	1.7675	1.4288	0.3572	16
31	24CIT 2K	4.3542	2.6253	0.5597	22

F ratio = 3.3764 f1 = 21 f2 = 15 p <0.01

Assuming Equal Variance : T = 3.5671 DF = 36 p = 0.001

Assuming Unequal Variance : T = 3.8958 DF = 34 p <0.001

Mann Whitney U Test.

Data from Column 29 and 31 including Row 1 to 134

Data set 1 24CIT 2 N = 16

Data set 2 24CIT 2K N = 22

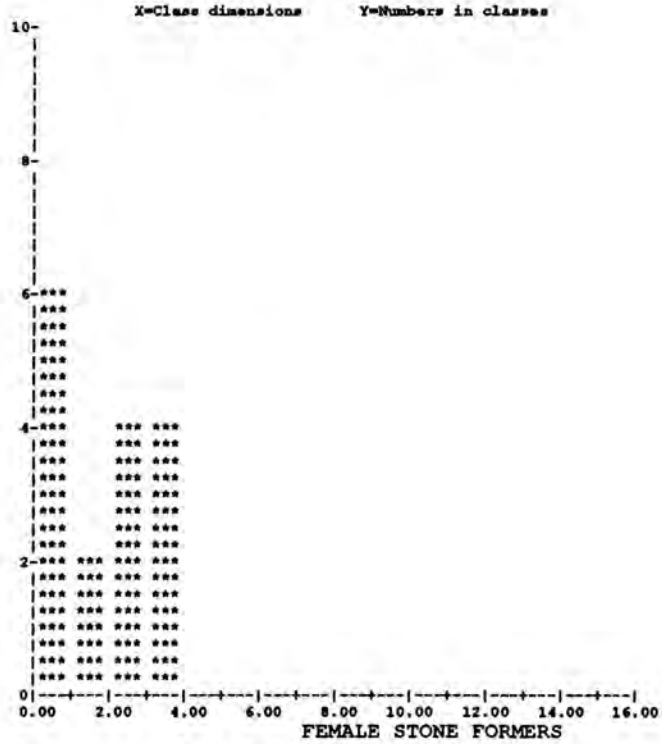
U = 71

Z = -3.1046 p = 0.002

FREQUENCY DISTRIBUTION

24 HR URINE CITRATE (MMOLS) -
After deletion of patients whose Calcium or Urate
or Oxalate was > (control mean + 2 S.D.)

Data from columns 29 to 29 and rows 1 to 134



FEMALE STONE FORMERS

Data from columns 31 to 31 and rows 1 to 134

FEMALE CONTROLS

24 HR URINE CITRATE/CREATININE RATIOS - (FEMALE STONE FORMERS and CONTROLS)
After deletion of patients whose Calcium or Urate
or Oxalate was > (control mean + 2 S.D.)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
45	CT/CR 2	0.2156	0.1742	0.0450	15
47	CT/CR 2K	0.4659	0.2730	0.0582	22

F ratio = 2.4557 f1 = 21 f2 = 14 p >=0.01 & p <0.05

Assuming Equal Variance : T = 3.1352 DF = 35 p = 0.003

Assuming Unequal Variance : T = 3.4030 DF = 35 p = 0.002

Mann Whitney U Test.

Data from Column 45 and 47 including Row 1 to 134

Data set 1 CT/CR 2 N = 15

Data set 2 CT/CR 2K N = 22

U = 73

Z = -2.8460 p = 0.004

FREQUENCY DISTRIBUTION

24 HR URINE CITRATE/CREATININE RATIO -
After deletion of patients whose Calcium or Urate
or Oxalate was > (control mean + 2 S.D.)

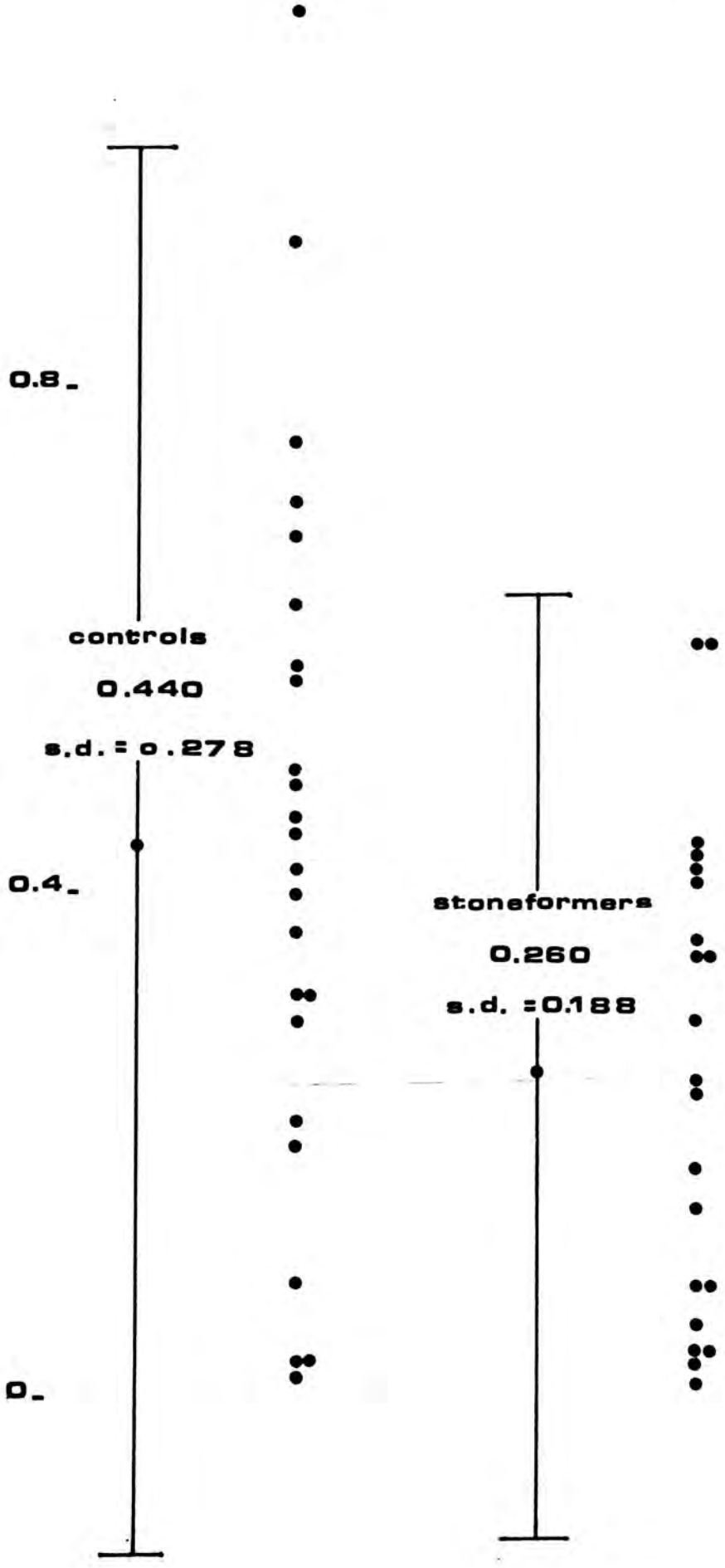
Data from columns 45 to 45 and rows 1 to 134

FEMALE STONE FORMERS

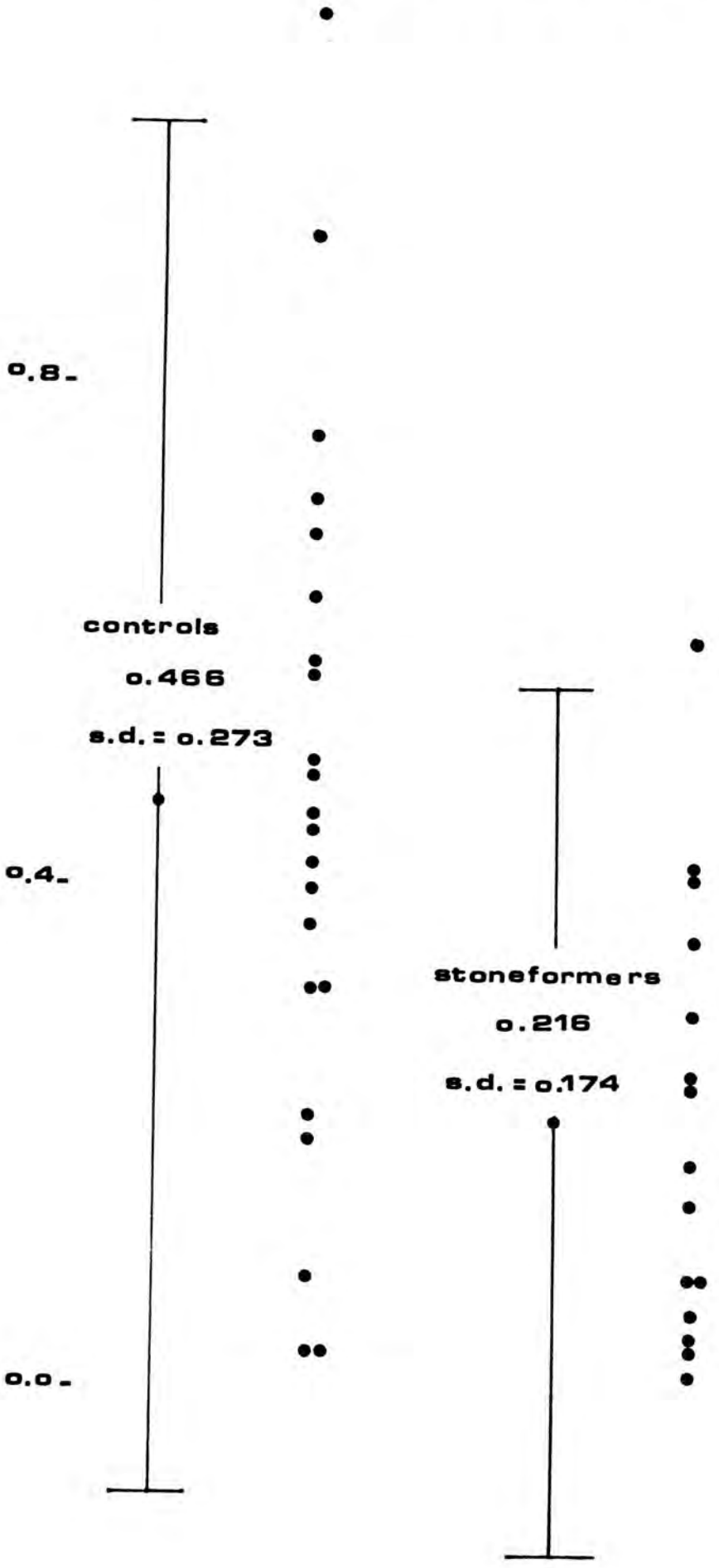
Data from columns 47 to 47 and rows 1 to 134

FEMALE CONTROLS

CITRATE/CREATININE RATIO (FEMALE STONE FORMERS and CONTROLS)



CITRATE/CREATININE RATIO (FEMALE STONE FORMERS and CONTROLS)
After exclusion of patients with urine Calcium or Urate
or Oxalate (> control mean + 2 S.D.)



24 HR URINE CITRATE (MMOLS) (FEMALE STONE FORMERS)
Pre-menopausal (< 50 yrs) v Postmenopausal (> 50 yrs)

Unpaired T-Test

Name	Mean	SD	N
24 CIT 2 (>50)	2.79	1.80	10
24 CIT 2 (<50)	2.36	2.33	11

p = 0.650

24 HR URINE CITRATE (MMOLS) (FEMALE CONTROLS)
Pre-menopausal (< 50 yrs) v Postmenopausal (> 50 yrs)

Unpaired T-Test

Name	Mean	SD	N
24 CIT 2K (>50)	4.25	2.20	10
24 CIT 2K (<50)	3.96	3.07	14

p = 0.789

24 HR URINE CITRATE/CALCIUM RATIO
MALES Vs CONTROLS
FEMALES Vs CONTROLS

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
48	CT/CA 1	0.3311	0.3511	0.0518	46
50	CT/CA 1K	0.5728	0.3545	0.0793	20

F ratio = 1.0193 f1 = 19 f2 = 45 p >=0.1

Assuming Equal Variance : T = 2.5630 DF = 64 p = 0.013

Assuming Unequal Variance : T = 2.5532 DF = 36 p = 0.015

Mann Whitney U Test.

Data from Column 48 and 50 including Row 1 to 134

Data set 1 CT/CA 1 N = 46

Data set 2 CT/CA 1K N = 20

U = 232

Z = -3.1812 p = 0.001

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
49	CT/CA 2	0.5010	0.3311	0.0740	20
51	CT/CA 2K	2.0729	2.1635	0.4416	24

F ratio = 42.6996 f1 = 23 f2 = 19 p <0.01

Assuming Equal Variance : T = 3.2119 DF = 42 p = 0.003

Assuming Unequal Variance : T = 3.5104 DF = 24 p = 0.002

Mann Whitney U Test.

Data from Column 49 and 51 including Row 1 to 134

Data set 1 CT/CA 2 N = 20

Data set 2 CT/CA 2K N = 24

U = 98

Z = -3.3470 p = < 0.001

24 HR URINE CITRATE/OXALATE RATIO
MALES Vs CONTROLS
FEMALES Vs CONTROLS

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
52	CT/OX 1	14.5578	11.4390	1.6685	47
54	CT/OX 1K	15.0263	16.3444	3.6547	20

F ratio = 2.0416 f1 = 19 f2 = 46 p >=0.01 & p <0.05

Assuming Equal Variance : T = 0.1343 DF = 65 p = 0.894

Assuming Unequal Variance : T = 0.1166 DF = 27 p = 0.908

Mann Whitney U Test.

Data from Column 52 and 54 including Row 1 to 134

Data set 1 CT/OX 1 N = 47

Data set 2 CT/OX 1K N = 20

U = 421

Z = -0.6714 p = 0.501

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
53	CT/OX 2	15.3965	12.7767	2.7881	21
55	CT/OX 2K	26.8951	24.8368	5.0698	24

F ratio = 3.7788 f1 = 23 f2 = 20 p <0.01

Assuming Equal Variance : T = 1.9101 DF = 43 p = 0.063

Assuming Unequal Variance : T = 1.9874 DF = 35 p = 0.055

Mann Whitney U Test.

Data from Column 53 and 55 including Row 1 to 134

Data set 1 CT/OX 2 N = 21

Data set 2 CT/OX 2K N = 24

U = 186

Z = -1.5016 p = 0.133

24 HR URINE CALCIUM OXALATE/CITRATE RATIO
MALES Vs CONTROLS
FEMALES Vs CONTROLS

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
60	CaO/Ci1	0.0907	0.1106	0.0163	46
62	CaO/Ci1K	0.0681	0.0956	0.0214	20

F ratio = 1.3361 f1 = 45 f2 = 19 p >=0.1

Assuming Equal Variance : T = 0.7949 DF = 64 p = 0.430

Assuming Unequal Variance : T = 0.8421 DF = 42 p = 0.405

Mann Whitney U Test.

Data from Column 60 and 62 including Row 1 to 134

Data set 1 CaO/Ci1 N = 46

Data set 2 CaO/Ci1K N = 20

U = 336

Z = -1.7301 p = 0.084

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
61	CaO/Ci2	0.0885	0.1133	0.0253	20
63	CaO/Ci2K	0.0525	0.1060	0.0216	24

F ratio = 1.1416 f1 = 19 f2 = 23 p >=0.1

Assuming Equal Variance : T = 1.0894 DF = 42 p = 0.282

Assuming Unequal Variance : T = 1.0828 DF = 39 p = 0.286

Mann Whitney U Test.

Data from Column 61 and 63 including Row 1 to 134

Data set 1 CaO/Ci2 N = 20

Data set 2 CaO/Ci2K N = 24

U = 122

Z = -2.7813 p = 0.005

24 HR URINE CALCIUM OXALATE/CREATININE RATIO
MALES Vs CONTROLS
FEMALES Vs CONTROLS

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
56	CaOx/Cr1	0.0120	0.0118	0.0017	46
58	CaO/Cr1K	0.0080	0.0050	0.0011	20

F ratio = 5.6741 f1 = 45 f2 = 19 p <0.01

Assuming Equal Variance : T = 1.4477 DF = 64 p = 0.153

Assuming Unequal Variance : T = 1.9282 DF = 64 p = 0.058

Mann Whitney U Test.

Data from Column 56 and 58 including Row 1 to 134

Data set 1 CaOx/Cr1 N = 46

Data set 2 CaO/Cr1K N = 20

U = 370

Z = -1.2557 p = 0.209

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
57	CaOx/Cr2	0.0105	0.0054	0.0012	20
59	CaO/Cr2K	0.0076	0.0060	0.0012	24

F ratio = 1.2011 f1 = 23 f2 = 19 p >=0.1

Assuming Equal Variance : T = 1.6626 DF = 42 p = 0.104

Assuming Unequal Variance : T = 1.6768 DF = 42 p = 0.101

Mann Whitney U Test.

Data from Column 57 and 59 including Row 1 to 134

Data set 1 CaOx/Cr2 N = 20

Data set 2 CaO/Cr2K N = 24

U = 160

Z = -1.8856 p = 0.059

24 HR URINE CALCIUM URATE/CITRATE RATIO
MALES Vs CONTROLS
FEMALES Vs CONTROLS

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
64	CU/CtCr1	2.2019	3.4328	0.5117	45
66	CU/C.C1K	0.8959	1.4315	0.3201	20

F ratio = 5.7507 f1 = 44 f2 = 19 p <0.01

Assuming Equal Variance : T = 1.6336 DF = 63 p = 0.107

Assuming Unequal Variance : T = 2.1636 DF = 63 p = 0.034

Mann Whitney U Test.

Data from Column 64 and 66 including Row 1 to 134

Data set 1 CU/CtCr1 N = 45

Data set 2 CU/C.C1K N = 20

U = 190

Z = -3.6955 p = < 0.001

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
65	CU/CtCr2	2.1718	3.7174	0.9016	17
67	CU/C.C2K	0.7526	1.5014	0.3065	24

F ratio = 6.1305 f1 = 16 f2 = 23 p <0.01

Assuming Equal Variance : T = 1.6923 DF = 39 p = 0.099

Assuming Unequal Variance : T = 1.4904 DF = 20 p = 0.152

Mann Whitney U Test.

Data from Column 65 and 67 including Row 1 to 134

Data set 1 CU/CtCr2 N = 17

Data set 2 CU/C.C2K N = 24

U = 95

Z = -2.8844 p = 0.004

Correlation 24 HR URINE CREATININE : 24 HR URINE CALCIUM (MALE STONE FORMERS)

	Column	Name
X data:	12	24CR 1
Y data:	16	24CA 1

Correlation= 0.153 N= 46

T = 1.0251 DF= 44 p= 0.311

Regression slope = 0.1152

Y-intercept = 6.8715

	Sums of squares	Variance
x	869.4722	19.3216
y	495.0158	11.0004
Cov	100.1970	2.2266

Variance about regression 10.9879

Correlation 24 HR URINE CREATININE : 24 HR URINE CALCIUM (FEMALE STONE FORMERS)

	Column	Name
X data:	14	24CR 2
Y data:	18	24CA 2

Correlation= 0.338 N= 20

T = 1.5213 DF= 18 p= 0.146

Regression slope = 0.3908

Y-intercept = 1.6209

	Sums of squares	Variance
x	157.3965	8.2840
y	210.9924	11.1049
Cov	61.5093	3.2373

Variance about regression 10.3864

Correlation 24 HR URINE CREATININE : 24 HR URINE CALCIUM (FEMALE CONTROLS)

	Column	Name
X data:	15	24CR 2 K
Y data:	19	24CA 2 K

Correlation=-0.188 N= 24

T = 0.8979 DF= 22 p= 0.379

Regression slope = -0.1511

Y-intercept = 4.2896

	Sums of squares	Variance
x	86.6800	3.7687
y	55.9978	2.4347
Cov	-13.0986	-0.5695

Variance about regression 2.4554

Correlation 24 HR URINE CREATININE : 24 HR URINE CALCIUM (MALE CONTROLS)

	Column	Name
X data:	13	24CR 1 K
Y data:	17	24CA 1 K

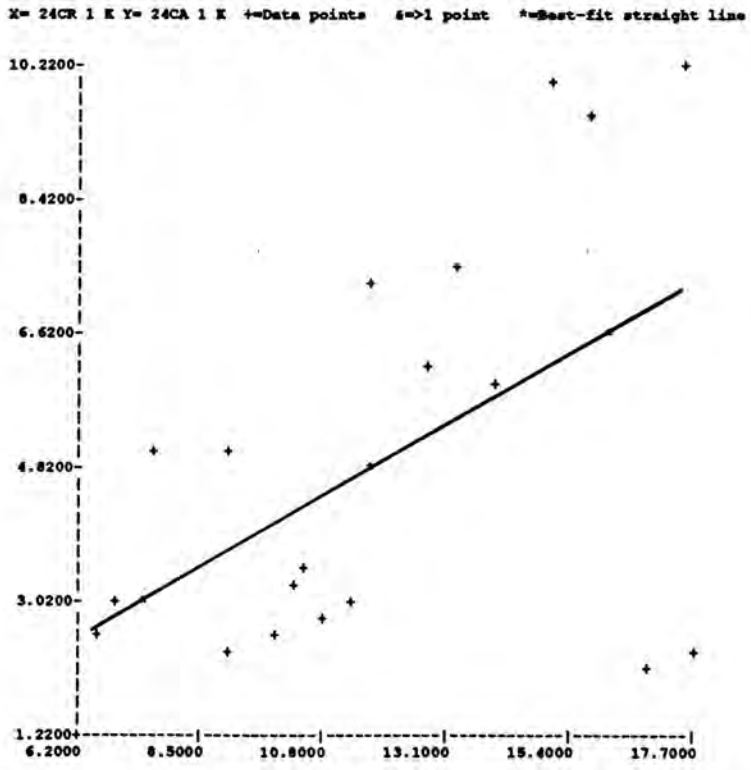
Correlation= 0.533 N= 20

T = 2.6759 DF= 18 p= 0.015

Regression slope = 0.4146

Y-intercept = -0.0385

	Sums of squares	Variance
x	251.4145	13.2323
y	151.8577	7.9925
Cov	104.2381	5.4862
Variance about regression		6.0355



Correlation 24 HR URINE URATE : 24 HR URINE CREATININE (MALE STONE FORMERS)

	Column	Name
X data:	20	24UR 1
Y data:	12	24CR 1

Correlation= 0.212 N= 45

T = 1.4241 DF= 43 p= 0.162

Regression slope = 0.5913

Y-intercept = 10.7396

	Sums of squares	Variance
x	110.9817	2.5223
y	861.6264	19.5824
Cov	65.6273	1.4915
Variance about regression		19.1353

Correlation 24 HR URINE URATE : 24 HR URINE CREATININE (MALE CONTROLS)

	Column	Name
X data:	21	24UR 1 K
Y data:	13	24CR 1 K

Correlation=-0.008 N= 20

T = 0.0337 DF= 18 p= 0.973

Regression slope = -0.0202

Y-intercept = 11.6864

	Sums of squares	Variance
x	38.8163	2.0430
y	251.4145	13.2323
Cov	-0.7853	-0.0413
Variance about regression		13.9666

Correlation 24 HR URINE URATE : 24 HR URINE CREATININE (FEMALE STONE FORMERS)

	Column	Name
X data:	22	24UR 2
Y data:	14	24CR 2

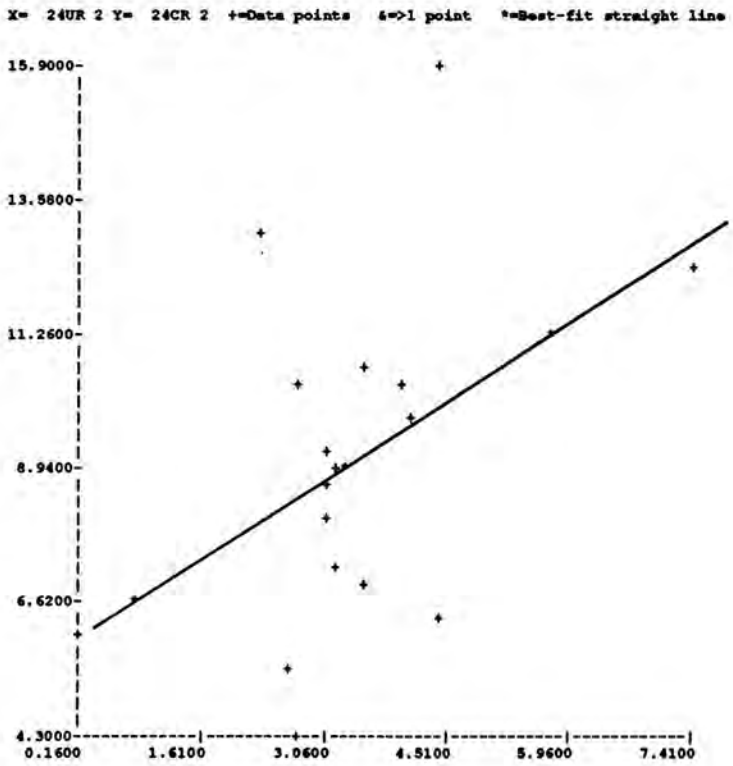
Correlation= 0.452 N= 17

T = 1.9616 DF= 15 p= 0.069

Regression slope = 0.9387

Y-intercept = 5.8354

	Sums of squares	Variance
x	32.7244	2.0453
y	141.2290	8.8268
Cov	30.7169	1.9198
Variance about regression		7.4931



Correlation 24 HR URINE URATE : 24 HR URINE CREATININE (FEMALE CONTROLS)

	Column	Name
X data:	23	24UR 2 K
Y data:	15	24CR 2 K

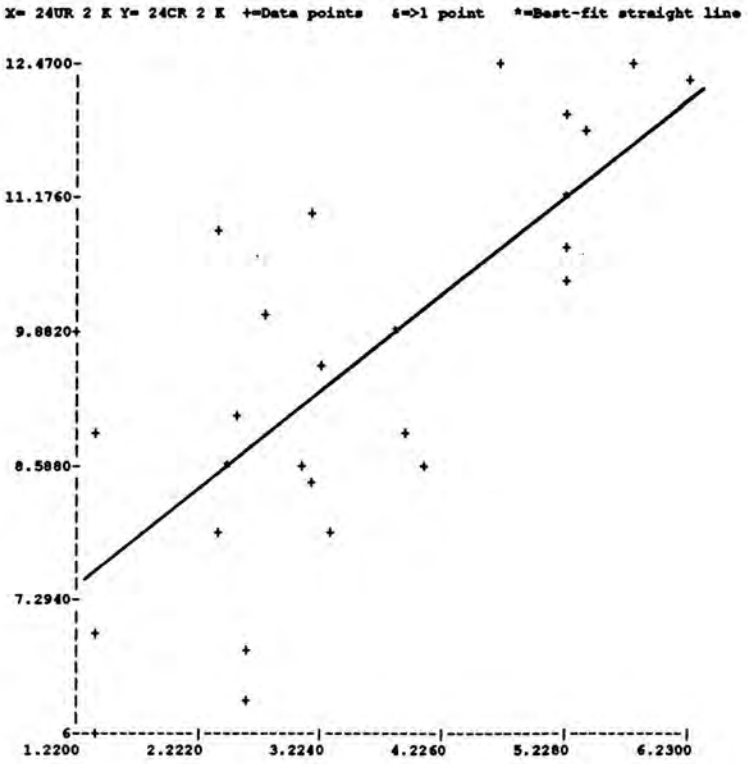
Correlation= 0.719 N= 24

T = 4.8454 DF= 22 p=<0.001

Regression slope = 0.9338

Y-intercept = 6.2897

	Sums of squares	Variance
x	51.3144	2.2311
y	86.6800	3.7687
Cov	47.9190	2.0834
Variance about regression		1.9060



Correlation 24 HR URINE CITRATE : 24 HR URINE CREATININE (MALE STONE FORMERS)

	Column	Name
X data:	28	24CIT 1
Y data:	12	24CR 1

Correlation= 0.106 N= 47

T = 0.7163 DF= 45 p= 0.478

Regression slope = 0.3537

Y-intercept = 12.7155

	Sums of squares	Variance
x	81.5772	1.7734
y	905.5385	19.6856
Cov	28.8573	0.6273

Variance about regression 19.8962

Correlation 24 HR URINE CITRATE : 24 HR URINE CREATININE (FEMALE CONTROLS)

	Column	Name
X data:	31	24CIT 2K
Y data:	15	24CR 2 K

Correlation= 0.093 N= 24

T = 0.4399 DF= 22 p= 0.664

Regression slope = 0.0674

Y-intercept = 9.2159

	Sums of squares	Variance
x	166.3122	7.2310
y	86.6800	3.7687
Cov	11.2127	0.4875

Variance about regression 3.9056

Correlation 24 HR URINE CITRATE : 24 HR URINE CREATININE (FEMALE STONE FORMERS)

	Column	Name
X data:	29	24CIT 2
Y data:	14	24CR 2

Correlation= 0.612 N= 21

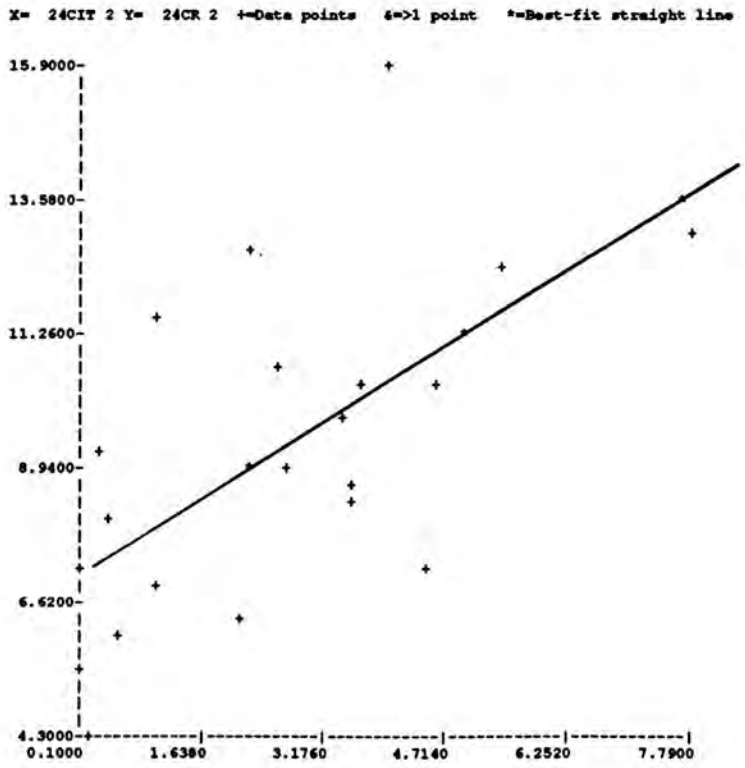
T = 3.3744 DF= 19 p= 0.003

Regression slope = 0.8510

Y-intercept = 7.0093

	Sums of squares	Variance
x	84.3855	4.2193
y	163.0875	8.1544
Cov	71.8120	3.5906

Variance about regression 5.3671



Correlation 24 HR URINE CITRATE : 24 HR URINE CREATININE (MALE CONTROLS)

	Column	Name
X data:	30	24CIT 1K
Y data:	13	24CR 1 K

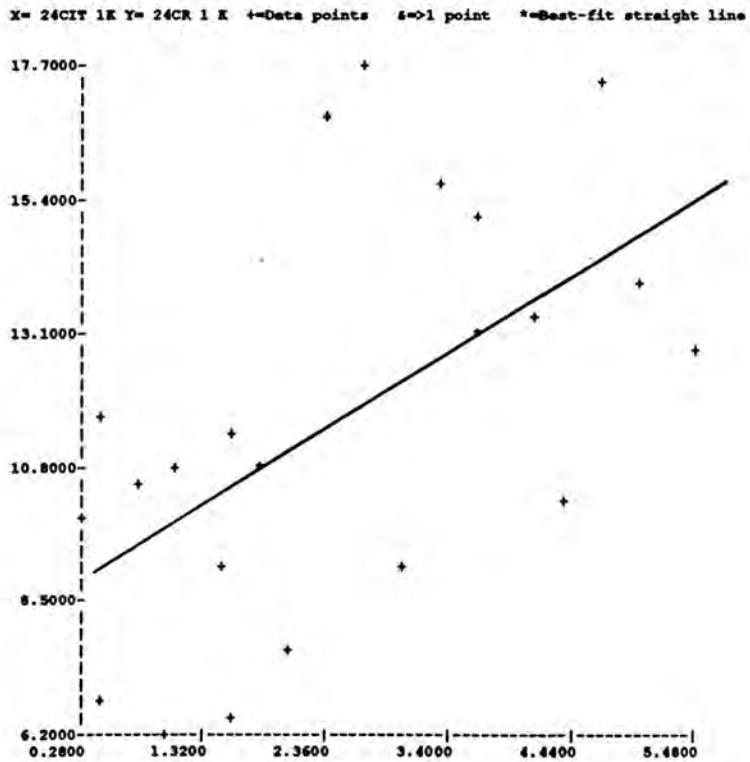
Correlation= 0.550 N= 20

T = 2.7932 DF= 18 p= 0.012

Regression slope = 1.2031

Y-intercept = 8.6733

	Sums of squares	Variance
x	52.5240	2.7644
y	251.4145	13.2323
Cov	63.1900	3.3258
Variance about regression		9.7440



Correlation 24 HR URINE CALCIUM : 24 HR URINE URATE (MALE CONTROLS)

	Column	Name
X data:	17	24CA 1 K
Y data:	21	24UR 1 K

Correlation= 0.076 N= 20

T = 0.3234 DF= 18 p= 0.750

Regression slope = 0.0384

Y-intercept = 2.5277

	Sums of squares	Variance
x	151.8577	7.9925
y	38.8163	2.0430
Cov	5.8354	0.3071
Variance about regression		2.1440

Correlation 24 HR URINE CALCIUM : 24 HR URINE URATE (FEMALE CONTROLS)

	Column	Name
X data:	19	24CA 2 K
Y data:	23	24UR 2 K

Correlation= 0.015 N= 24

T = 0.0685 DF= 22 p= 0.946

Regression slope = 0.0140

Y-intercept = 3.3880

	Sums of squares	Variance
x	55.9978	2.4347
y	51.3144	2.2311
Cov	0.7832	0.0341
Variance about regression		2.3320

Correlation 24 HR URINE CALCIUM : 24 HR URINE URATE (MALE STONE FORMERS)

	Column	Name
X data:	16	24CA 1
Y data:	20	24UR 1

Correlation= 0.339 N= 56

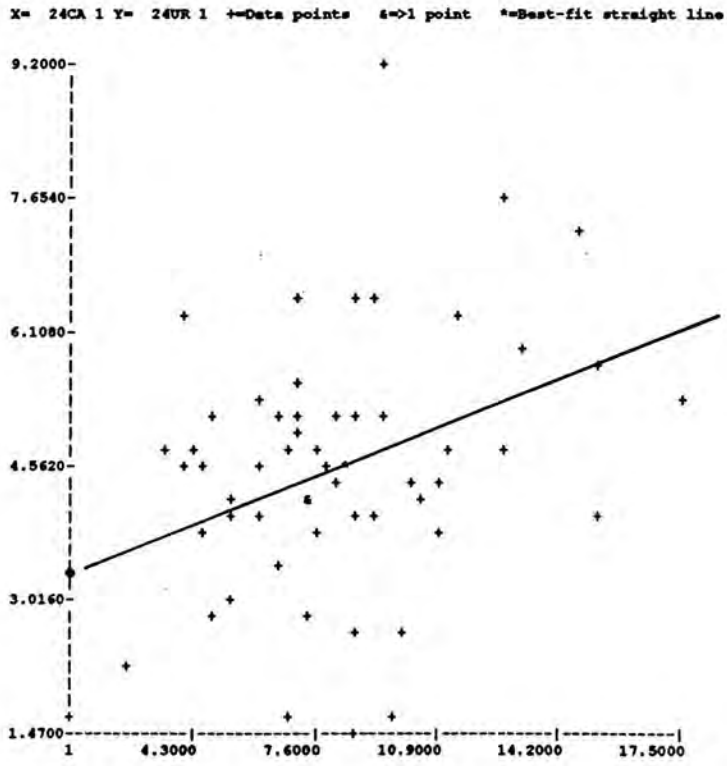
T = 2.6462 DF= 54 p= 0.011

Regression slope = 0.1524

Y-intercept = 3.3003

	Sums of squares	Variance
x	611.1042	11.1110
y	123.6481	2.2481
Cov	93.1335	1.6933

Variance about regression 2.0269



Correlation CALCIUM/CREATININE RATIO : URATE/CREATININE RATIO (MALE STONE FORMERS)

	Column	Name
X data:	32	CA 1/CR
Y data:	36	UR 1/CR

Correlation= 0.675 N= 45

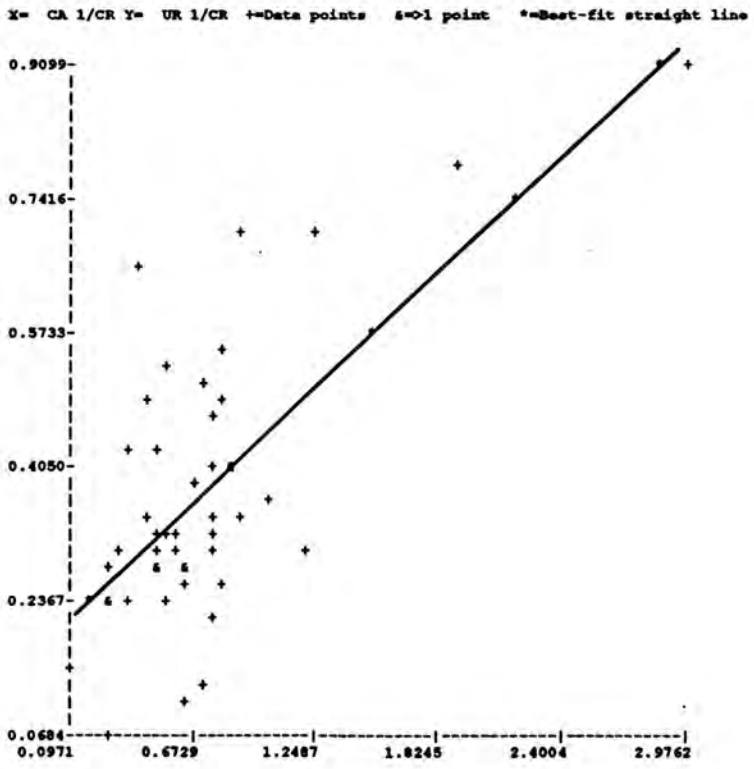
T = 6.0017 DF= 43 p=<0.001

Regression slope = 0.2555

Y-intercept = 0.1871

	Sums of squares	Variance
x	9.4183	0.2141
y	1.3492	0.0307
Cov	2.4067	0.0547

Variance about regression 0.0171



Correlation 24 HR URINE CALCIUM : 24 HR URINE URATE (FEMALE STONE FORMERS)

	Column	Name
X data:	18	24CA 2
Y data:	22	24UR 2

Correlation= 0.629 N= 25

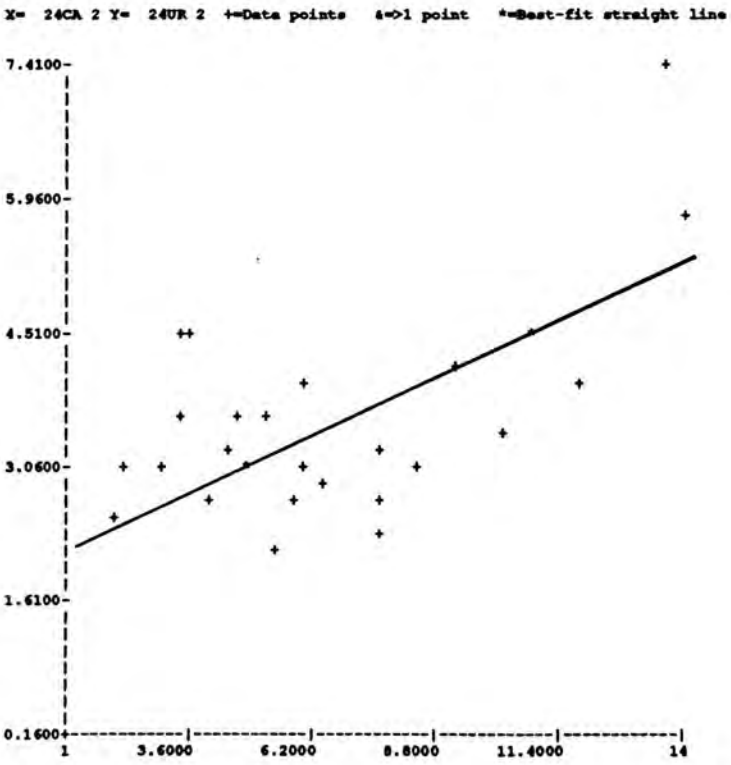
T = 3.8816 DF= 23 p=<0.001

Regression slope = 0.2368

Y-intercept = 1.9339

	Sums of squares	Variance
x	288.6002	12.0250
y	40.8998	1.7042
Cov	68.3508	2.8480

Variance about regression 1.0744



Correlation CALCIUM/CREATININE RATIO : URATE/CREATININE RATIO
(FEMALE STONE FORMERS)

	Column	Name
X data:	33	CA 2/CR
Y data:	37	UR 2/CR

Correlation= 0.517 N= 17

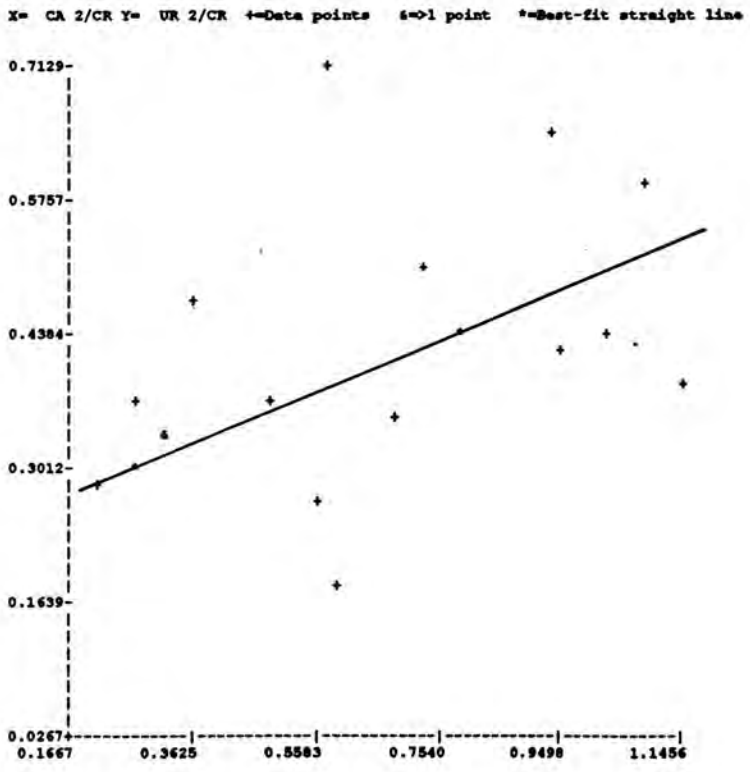
T = 2.3383 DF= 15 p= 0.034

Regression slope = 0.2694

Y-intercept = 0.2289

	Sums of squares	Variance
x	1.6502	0.1031
y	0.4482	0.0280
Cov	0.4445	0.0278

Variance about regression 0.0219



Correlation 24 HR URINE CALCIUM : 24 HR URINE CITRATE (MALE STONE FORMERS)

	Column	Name
X data:	16	24CA 1
Y data:	28	24CIT 1

Correlation= 0.040 N= 46

T = 0.2681 DF= 44 p= 0.790

Regression slope = 0.0157

Y-intercept = 2.1950

	Sums of squares	Variance
x	495.0158	11.0004
y	74.6368	1.6586
Cov	7.7632	0.1725
Variance about regression		1.6935

Correlation 24 HR URINE CALCIUM : 24 HR URINE CITRATE (FEMALE CONTROLS)

	Column	Name
X data:	19	24CA 2 K
Y data:	31	24CIT 2K

Correlation=-0.156 N= 24

T = 0.7386 DF= 22 p= 0.468

Regression slope = -0.2681

Y-intercept = 4.8439

	Sums of squares	Variance
x	55.9978	2.4347
y	166.3122	7.2310
Cov	-15.0110	-0.6527
Variance about regression		7.3767

Correlation 24 HR URINE CALCIUM : 24 HR URINE CITRATE (MALE CONTROLS)

	Column	Name
X data:	17	24CA 1 K
Y data:	30	24CIT 1K

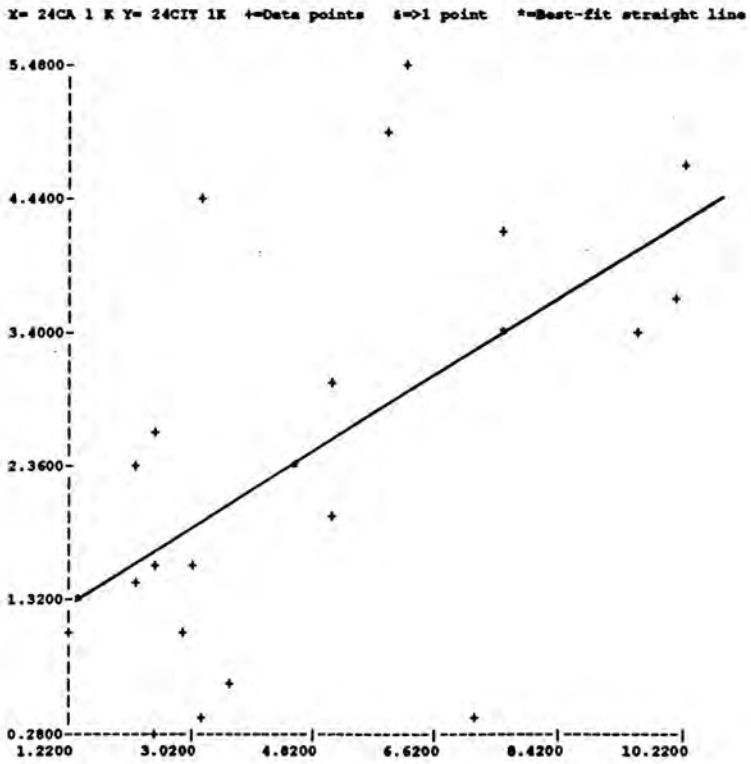
Correlation= 0.569 N= 20

T = 2.9332 DF= 18 p= 0.009

Regression slope = 0.3345

Y-intercept = 0.8589

	Sums of squares	Variance
x	151.8577	7.9925
y	52.5240	2.7644
Cov	50.7888	2.6731
Variance about regression		1.9743



Correlation 24 HR URINE CALCIUM : 24 HR URINE CITRATE (FEMALE STONE FORMERS)

	Column	Name
X data:	18	24CA 2
Y data:	29	24CIT 2

Correlation= 0.721 N= 20

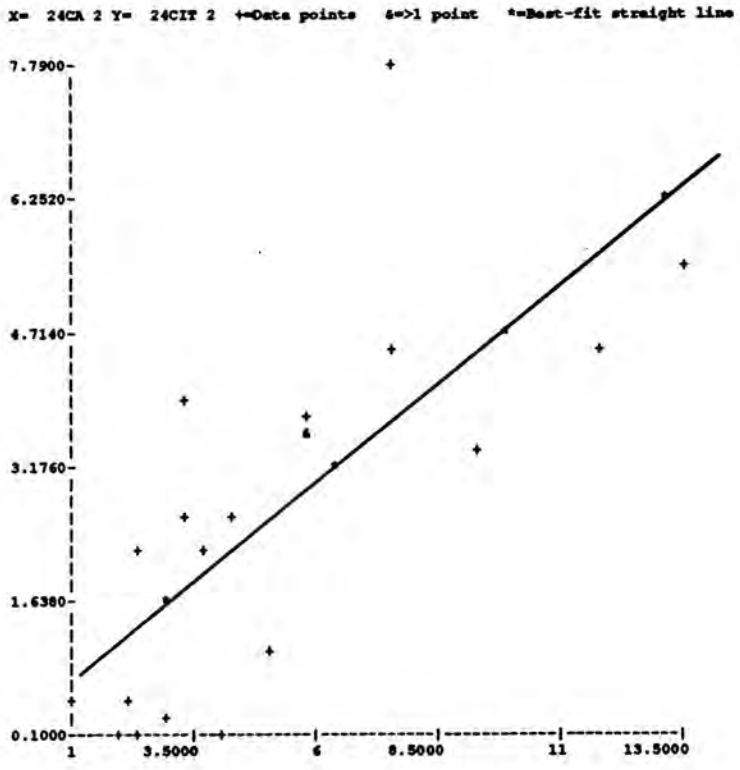
T = 4.4157 DF= 18 p=<0.001

Regression slope = 0.4497

Y-intercept = 0.3150

	Sums of squares	Variance
x	210.9924	11.1049
y	82.0708	4.3195
Cov	94.8903	4.9942

Variance about regression 2.1886



Correlation CALCIUM/CREATININE RATIO : CITRATE/CREATININE RATIO
(FEMALE CONTROLS)

	Column	Name
X data:	35	CA 2K/CR
Y data:	47	CT/CR 2K

Correlation=-0.029 N= 24

T = 0.1356 DF= 22 p= 0.893

Regression slope = -0.0379

Y-intercept = 0.4525

	Sums of squares	Variance
x	1.0335	0.0449
y	1.7793	0.0774
Cov	-0.0392	-0.0017

Variance about regression 0.0808

Correlation CALCIUM/CREATININE RATIO : CITRATE/CREATININE RATIO
(MALE STONE FORMERS)

	Column	Name
X data:	32	CA 1/CR
Y data:	44	CT/CR 1

Correlation= 0.077 N= 46

T = 0.5133 DF= 44 p= 0.610

Regression slope = 0.0222

Y-intercept = 0.1753

	Sums of squares	Variance
x	9.4491	0.2100
y	0.7818	0.0174
Cov	0.2097	0.0047

Variance about regression 0.0177

Correlation CALCIUM/CREATININE RATIO : CITRATE/CREATININE RATIO
(MALE CONTROLS)

	Column	Name
X data:	34	CA 1K/CR
Y data:	46	CT/CR 1K

Correlation= 0.337 N= 20

T = 1.5211 DF= 18 p= 0.146

Regression slope = 0.2324

Y-intercept = 0.1114

	Sums of squares	Variance
x	0.6028	0.0317
y	0.2857	0.0150
Cov	0.1401	0.0074

Variance about regression 0.0141

Correlation CALCIUM/CREATININE RATIO : CITRATE/CREATININE RATIO
(FEMALE STONE FORMERS)

	Column	Name
X data:	33	CA 2/CR
Y data:	45	CT/CR 2

Correlation= 0.581 N= 20

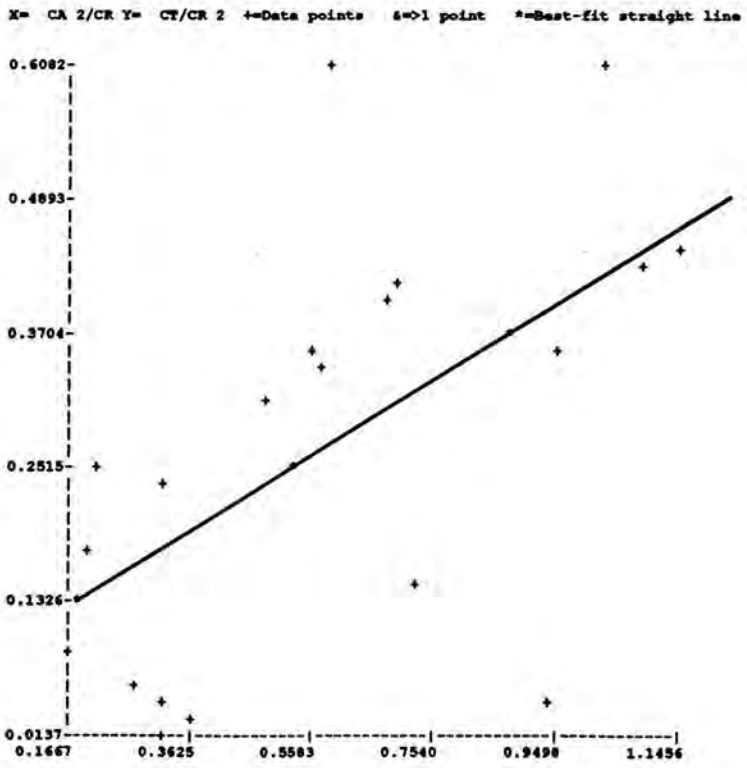
T = 3.0259 DF= 18 p= 0.007

Regression slope = 0.3459

Y-intercept = 0.0676

	Sums of squares	Variance
x	1.8999	0.1000
y	0.6740	0.0355
Cov	0.6571	0.0346

Variance about regression 0.0248



Correlation 24 HR URINE CITRATE : 24 HR URINE URATE (MALE STONE FORMERS)

	Column	Name
X data:	28	24CIT 1
Y data:	20	24UR 1

Correlation=-0.059 N= 45

T = 0.3870 DF= 43 p= 0.701

Regression slope = -0.0722

Y-intercept = 4.6115

	Sums of squares	Variance
x	73.8932	1.6794
y	110.9817	2.5223
Cov	-5.3352	-0.1213

Variance about regression 2.5720

Correlation 24 HR URINE CITRATE : 24 HR URINE URATE (MALE CONTROLS)

	Column	Name
X data:	30	24CIT 1K
Y data:	21	24UR 1 K

Correlation= 0.233 N= 20

T = 1.0178 DF= 18 p= 0.322

Regression slope = 0.2005

Y-intercept = 2.2184

	Sums of squares	Variance
x	52.5240	2.7644
y	38.8163	2.0430
Cov	10.5334	0.5544

Variance about regression 2.0391

Correlation CITRATE/CREATININE RATIO : URATE/CREATININE RATIO
(MALE STONE FORMERS)

	Column	Name
X data:	44	CT/CR 1
Y data:	36	UR 1/CR

Correlation= 0.076 N= 45

T = 0.4992 DF= 43 p= 0.620

Regression slope = 0.0997

Y-intercept = 0.3474

	Sums of squares	Variance
x	0.7818	0.0178
y	1.3492	0.0307
Cov	0.0780	0.0018
Variance about regression		0.0312

Correlation CITRATE/CREATININE RATIO : URATE/CREATININE RATIO
(MALE CONTROLS)

	Column	Name
X data:	46	CT/CR 1K
Y data:	38	UR 1K/CR

Correlation= 0.190 N= 20

T = 0.8227 DF= 18 p= 0.421

Regression slope = 0.2156

Y-intercept = 0.2060

	Sums of squares	Variance
x	0.2857	0.0150
y	0.3665	0.0193
Cov	0.0616	0.0032
Variance about regression		0.0196

Correlation 24 HR URINE CITRATE : 24 HR URINE URATE (FEMALE STONE FORMERS)

	Column	Name
X data:	29	24CIT 2
Y data:	22	24UR 2

Correlation= 0.369 N= 17

T = 1.5388 DF= 15 p= 0.145

Regression slope = 0.2447

Y-intercept = 2.7108

	Sums of squares	Variance
x	74.5100	4.6569
y	32.7244	2.0453
Cov	18.2332	1.1396
Variance about regression		1.8842

Correlation 24 HR URINE CITRATE : 24 HR URINE URATE (FEMALE CONTROLS)

	Column	Name
X data:	31	24CIT 2K
Y data:	23	24UR 2 K

Correlation= 0.092 N= 24

T = 0.4347 DF= 22 p= 0.668

Regression slope = 0.0513

Y-intercept = 3.2189

	Sums of squares	Variance
x	166.3122	7.2310
y	51.3144	2.2311
Cov	8.5243	0.3706
Variance about regression		2.3126

Correlation CITRATE/CREATININE RATIO : URATE/CREATININE RATIO
(FEMALE STONE FORMERS)

	Column	Name
X data:	45	CT/CR 2
Y data:	37	UR 2/CR

Correlation=-0.043 N= 17

T = 0.1652 DF= 15 p= 0.871

Regression slope = -0.0375

Y-intercept = 0.4039

	Sums of squares	Variance
x	0.5776	0.0361
y	0.4482	0.0280
Cov	-0.0217	-0.0014
Variance about regression		0.0298

Correlation CITRATE/CREATININE RATIO : URATE/CREATININE RATIO
(FEMALE CONTROLS)

	Column	Name
X data:	47	CT/CR 2K
Y data:	39	UR 2K/CR

Correlation=-0.028 N= 24

T = 0.1334 DF= 22 p= 0.895

Regression slope = -0.0115

Y-intercept = 0.3590

	Sums of squares	Variance
x	1.7793	0.0774
y	0.2934	0.0128
Cov	-0.0205	-0.0009
Variance about regression		0.0133

ARTERIAL BLOOD GASES IN STONEFORMERS (MALE and FEMALE)

ref range H+ 36-44 mmol/L
pCO_2 4.4-6.1 kPA
HCO_3 21-27.5 mmol/L
pO_2 12-15 kPA

(MALE and FEMALE COMBINED)

Calculations by Column:Including Rows 1 - 134

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
2	H+	40.48	2.89	0.45	7.14	42

Calculations by Column:Including Rows 1 - 134

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
3	pCO2	5.12	0.44	0.07	8.58	42

Calculations by Column:Including Rows 1 - 134

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
4	HCO3	23.80	1.85	0.29	7.79	42

Calculations by Column:Including Rows 1 - 134

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
5	pO2	11.63	1.60	0.25	13.78	42

(ARTERIAL HCO_3 FEMALE)

Calculations by Column:Including Rows 1 - 29

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
4	HCO3	23.0833	1.8195	0.5252	7.8823	12

(ARTERIAL HCO_3 MALE)

Calculations by Column:Including Rows 56 - 113

Column	Name	Mean	S.D.	S.E.M.	Coef.V	N
4	HCO3	24.0833	1.8198	0.3323	7.5564	30

Correlation 24 HR URINE CITRATE : ARTERIAL H+ (MALE STONE FORMERS)

	Column	Name
X data:	2	H+
Y data:	28	24CIT 1

Correlation=-0.209 N= 26

T = 1.0460 DF= 24 p= 0.306

Regression slope = -0.1030

Y-intercept = 6.4030

	Sums of squares	Variance
x	136.3462	5.4538
y	33.1787	1.3271
Cov	-14.0437	-0.5617

Variance about regression 1.3222

Correlation CITRATE/CREATININE RATIO : ARTERIAL H+ (MALE STONE FORMERS)

	Column	Name
X data:	2	H+
Y data:	44	CT/CR 1

Correlation=-0.166 N= 26

T = 0.8260 DF= 24 p= 0.417

Regression slope = -0.0069

Y-intercept = 0.4565

	Sums of squares	Variance
x	136.3462	5.4538
y	0.2372	0.0095
Cov	-0.9455	-0.0378

Variance about regression 0.0096

Correlation 24 HR URINE CITRATE : ARTERIAL H+ (FEMALE STONE FORMERS)

	Column	Name
X data:	2	H+
Y data:	29	24CIT 2

Correlation=-0.058 N= 12

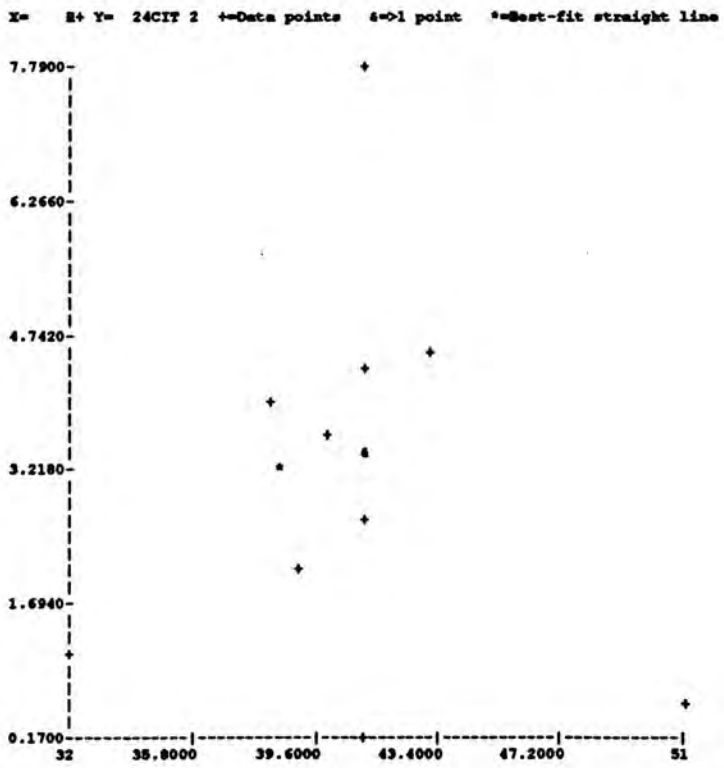
T = 0.1836 DF= 10 p= 0.858

Regression slope = -0.0284

Y-intercept = 4.3109

	Sums of squares	Variance
x	198.2500	18.0227
y	47.5377	4.3216
Cov	-5.6275	-0.5116

Variance about regression 4.7378



Correlation CITRATE/CREATININE RATIO : ARTERIAL H+ (FEMALE STONE FORMERS)

	Column	Name
X data:	2	H+
Y data:	45	CT/CR 2

Correlation= 0.013 N= 12

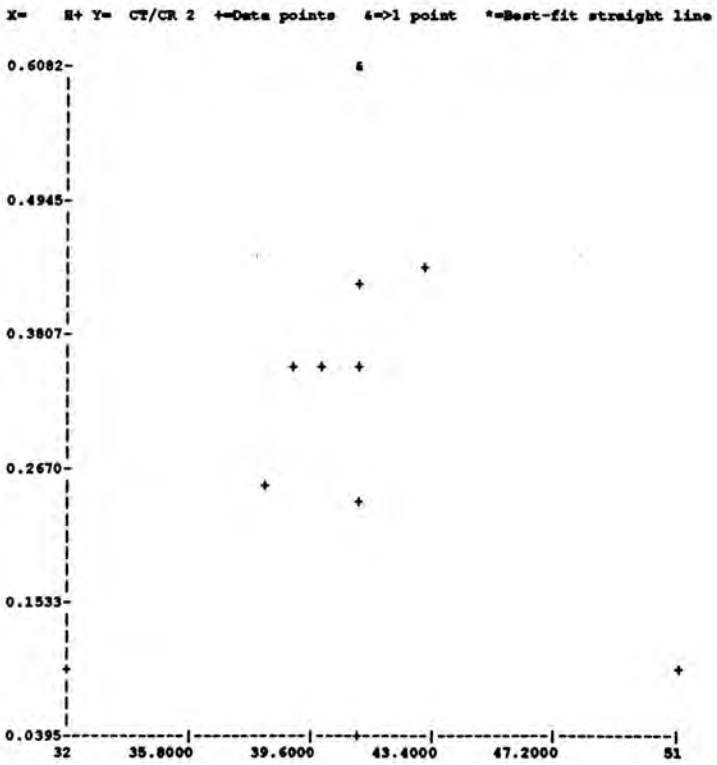
T = 0.0408 DF= 10 p= 0.968

Regression slope = 0.0006

Y-intercept = 0.2969

	Sums of squares	Variance
x	198.2500	18.0227
y	0.3830	0.0348
Cov	0.1124	0.0102

Variance about regression 0.0383



Correlation 24 HR URINE CITRATE : ARTERIAL HCO, (MALE STONE FORMERS)

	Column	Name
X data:	4	HCO3
Y data:	28	24CIT 1

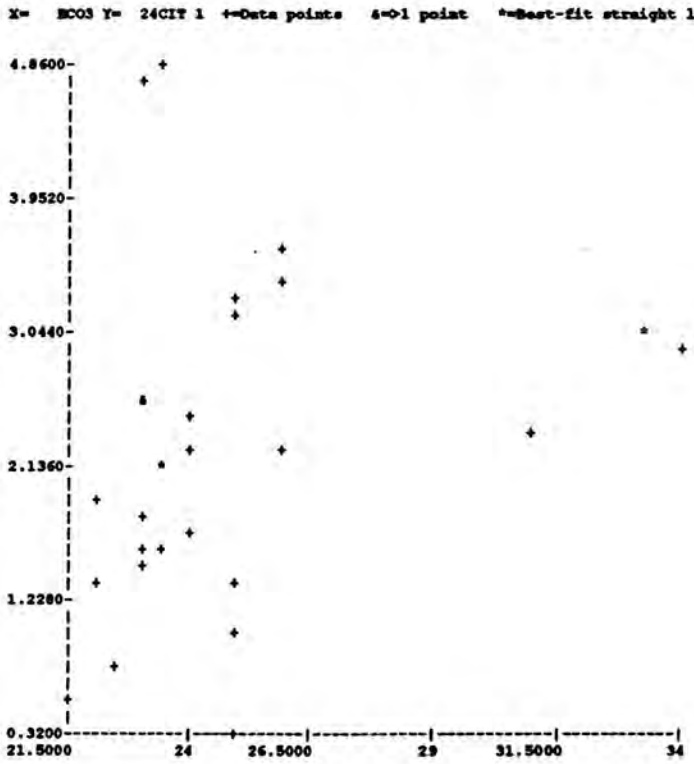
Correlation= 0.219 N= 26

T = 1.0977 DF= 24 p= 0.283

Regression slope = 0.0927

Y-intercept = -0.0288

	Sums of squares	Variance
x	184.4615	7.3785
y	33.1787	1.3271
Cov	17.1044	0.6842
Variance about regression		1.3164



Correlation CITRATE/CREATININE RATIO : ARTERIAL HCO_3 (MALE STONE FORMERS)

	Column	Name
X data:	4	HCO3
Y data:	44	CT/CR 1

Correlation= 0.280 N= 26

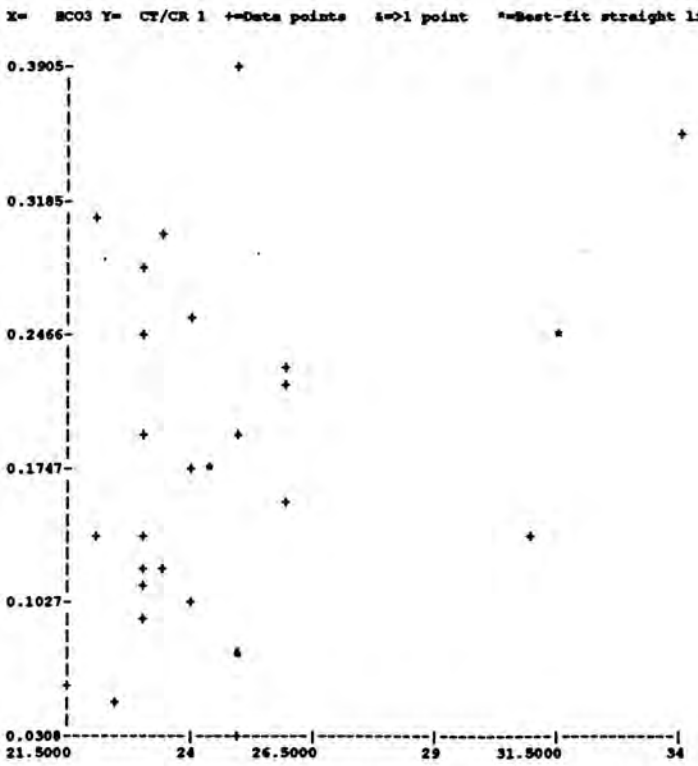
T = 1.4299 DF= 24 p= 0.166

Regression slope = 0.0100

Y-intercept = -0.0696

	Sums of squares	Variance
x	184.4615	7.3785
y	0.2372	0.0095
Cov	1.8534	0.0741

Variance about regression 0.0091



Correlation 24 HR URINE CITRATE : ARTERIAL HCO_3 (FEMALE STONE FORMERS)

	Column	Name
X data:	4	HCO3
Y data:	29	24CIT 2

Correlation= 0.587 N= 12

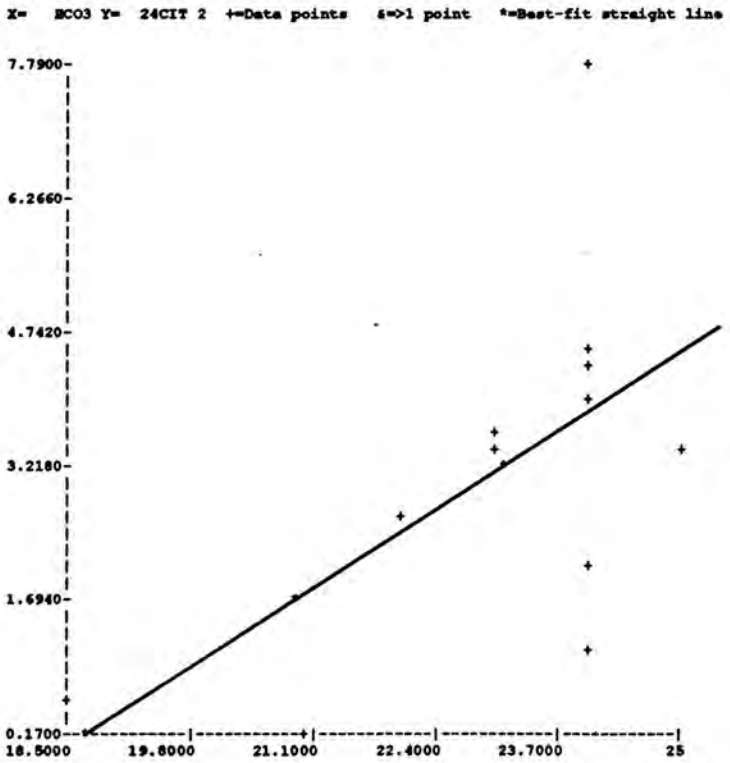
T = 2.2923 DF= 10 p= 0.045

Regression slope = 0.6818

Y-intercept = -12.5548

	Sums of squares	Variance
x	35.2292	3.2027
y	47.5377	4.3216
Cov	24.0179	2.1834

Variance about regression 3.1163



Correlation CITRATE/CREATININE RATIO : ARTERIAL HCO_3 (FEMALE STONE FORMERS)

	Column	Name
X data:	4	HCO3
Y data:	45	CT/CR 2

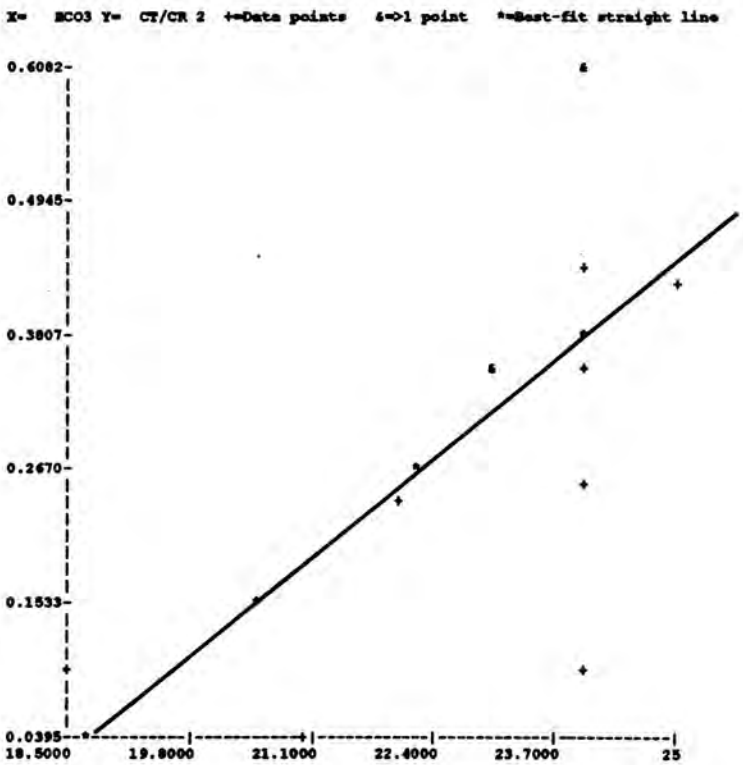
Correlation= 0.621 N= 12

T = 2.5083 DF= 10 p= 0.031

Regression slope = 0.0648

Y-intercept = -1.1730

	Sums of squares	Variance
x	35.2292	3.2027
y	0.3830	0.0348
Cov	2.2827	0.2075
Variance about regression		0.0235



Correlation CITRATE/CREATININE RATIO : ARTERIAL HCO, (FEMALE STONE FORMERS)
(After deletion of CT/CR values < 0.16)

	Column	Name
X data:	4	HCO3
Y data:	45	CT/CR 2

Correlation= 0.468 N= 9

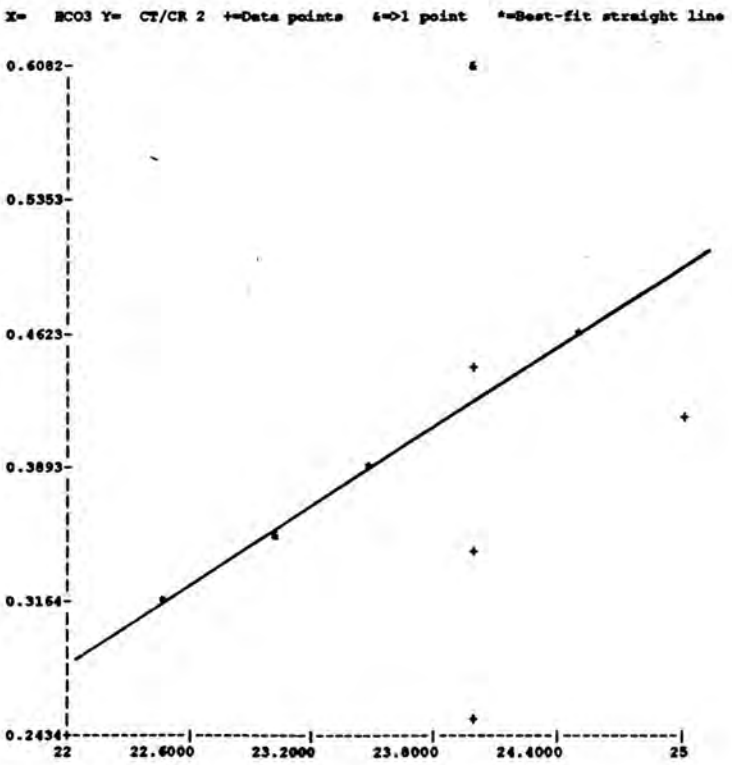
T = 1.4021 DF= 7 p= 0.204

Regression slope = 0.0718

Y-intercept = -1.2974

	Sums of squares	Variance
x	6	0.7500
y	0.1410	0.0176
Cov	0.4308	0.0538

Variance about regression 0.0157



Correlation 24 HR URINE CITRATE : ARTERIAL HCO_3 (MALE STONE FORMERS)
After exclusion of patients with urine Calcium or Urate
or Oxalate (> control mean + 2 S.D.)

	Column	Name
X data:	4	HCO3
Y data:	37	24CIT 1

Correlation= 0.133 N= 16

T = 0.5027 DF= 14 p= 0.623

Regression slope = 0.1313

Y-intercept = -1.0253

	Sums of squares	Variance
x	20.4844	1.3656
y	19.9156	1.3277
Cov	2.6894	0.1793
Variance about regression		1.3973

Correlation CITRATE/CREATININE RATIO : ARTERIAL HCO_3 (MALE STONE FORMERS)
After exclusion of patients with urine Calcium or Urate
or Oxalate (> control mean + 2 S.D.)

	Column	Name
X data:	4	HCO3
Y data:	41	CT/CR 1

Correlation= 0.039 N= 15

T = 0.1393 DF= 13 p= 0.891

Regression slope = 0.0018

Y-intercept = 0.1152

	Sums of squares	Variance
x	60.9333	4.3524
y	0.1393	0.0100
Cov	0.1125	0.0080
Variance about regression		0.0107

Correlation 24 HR URINE CITRATE : ARTERIAL HCO_3 (FEMALE STONE FORMERS)
After exclusion of patients with urine Calcium or Urate
or Oxalate (> control mean + 2 S.D.)

	Column	Name
X data:	4	HCO3
Y data:	38	24CIT 2

Correlation= 0.654 N= 8

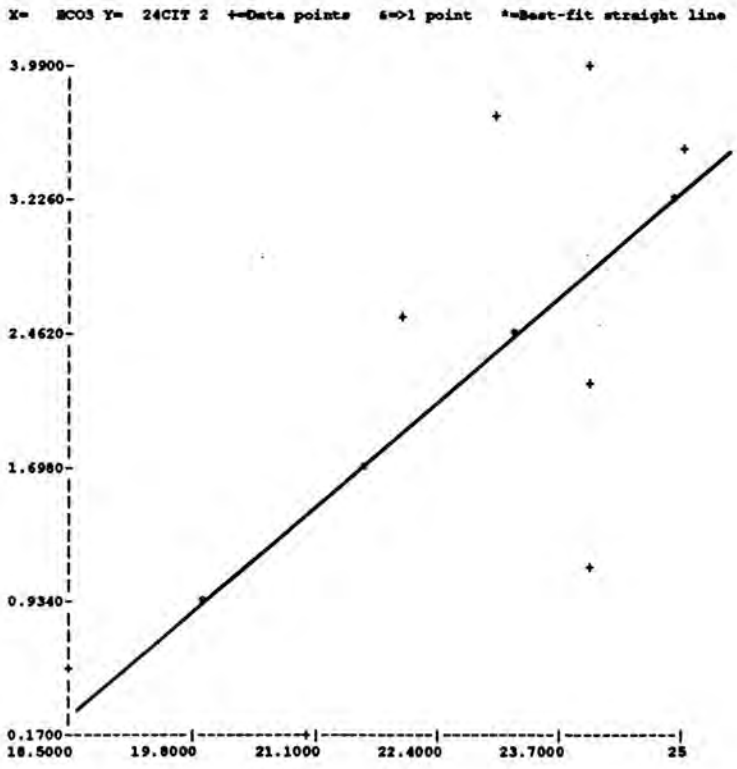
T = 2.1158 DF= 6 p= 0.079

Regression slope = 0.4562

Y-intercept = -8.1377

	Sums of squares	Variance
x	31.4688	4.4955
y	15.3279	2.1897
Cov	14.3562	2.0509

Variance about regression 1.4631



Correlation CITRATE/CREATININE RATIO : ARTERIAL HCO+ (FEMALE STONE FORMERS)
After exclusion of patients with urine Calcium or Urate
or Oxalate (> control mean + 2 S.D.)

	Column	Name
X data:	4	HCO3
Y data:	42	CT/CR 2

Correlation= 0.236 N= 7

T = 0.5436 DF= 5 p= 0.610

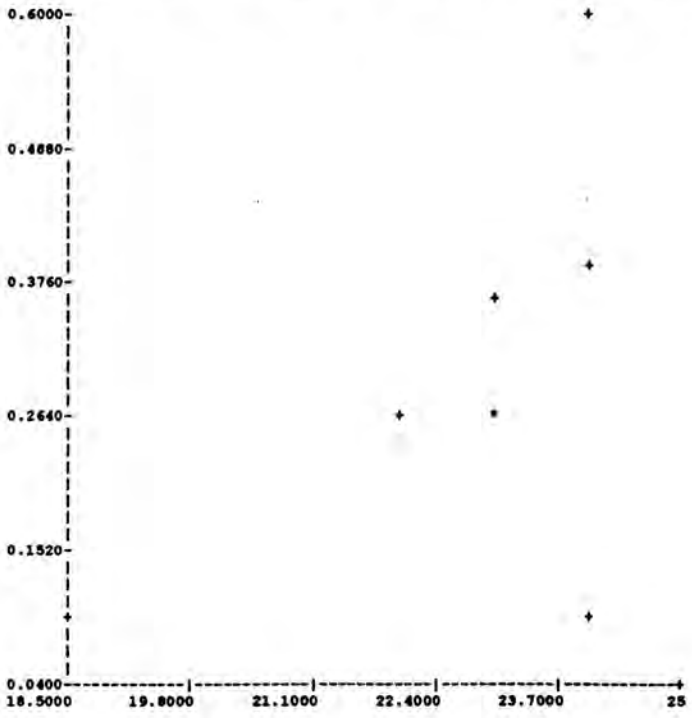
Regression slope = 0.0222

Y-intercept = -0.2471

	Sums of squares	Variance
x	28.2143	4.7024
y	0.2487	0.0414
Cov	0.6257	0.1043

Variance about regression 0.0470

X= HCO3 Y= CT/CR 2 +=Data points &=>1 point *=Best-fit straight line



SERUM CREATININE (MALE STONE FORMERS Vs FEMALE STONE FORMERS)

Unpaired T-Test

Set	Name	Mean	SD	SEM	N
7	S.CREAT1	103.38	16.12	2.85	32
8	S.CREAT2	82	13.26	3.68	13

F ratio = 1.4782 f1 = 31 f2 = 12 p >=0.1

Assuming Equal Variance : T = 4.2264 DF = 43 p <0.001

Assuming Unequal Variance : T = 4.5941 DF = 27 p <0.001

Correlation SERUM CREATININE : 24 HR URINE CITRATE (MALE STONE FORMERS)

	Column	Name
X data:	7	SE.CREAT
Y data:	28	24CIT 1

Correlation=-0.261 N= 26

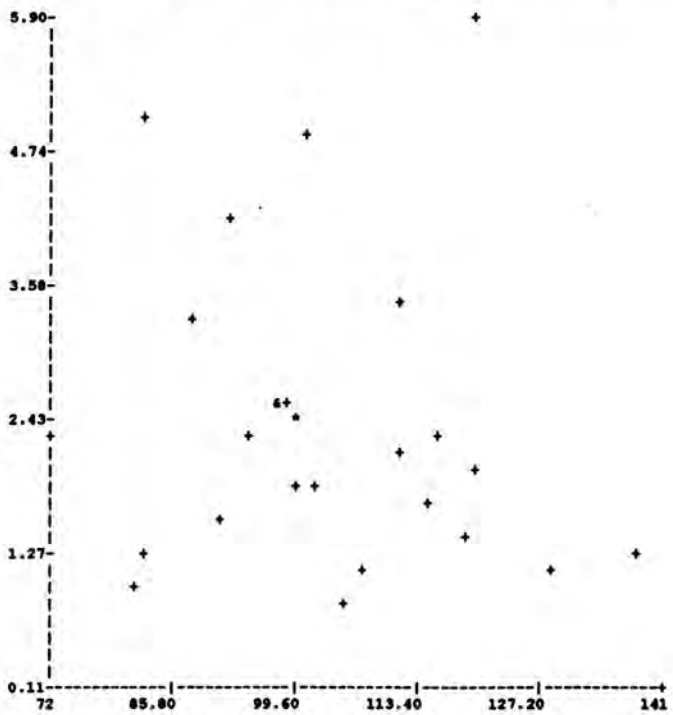
T = 1.33 DF= 24 p= 0.197

Regression slope = -0.02

Y-intercept = 4.54

	Sums of squares	Variance
x	7349.54	293.98
y	48.39	1.94
Cov	-155.77	-6.23
Variance about regression		1.88

X= SE.CREAT Y= 24CIT 1 +=Data points &=>1 point *=Best-fit straight line



Correlation SERUM CREATININE : CITRATE/CREATININE RATIO (MALE STONE FORMERS)

	Column	Name
X data:	7	SE.CREAT
Y data:	44	CT/CR 1

Correlation=-0.171 N= 26

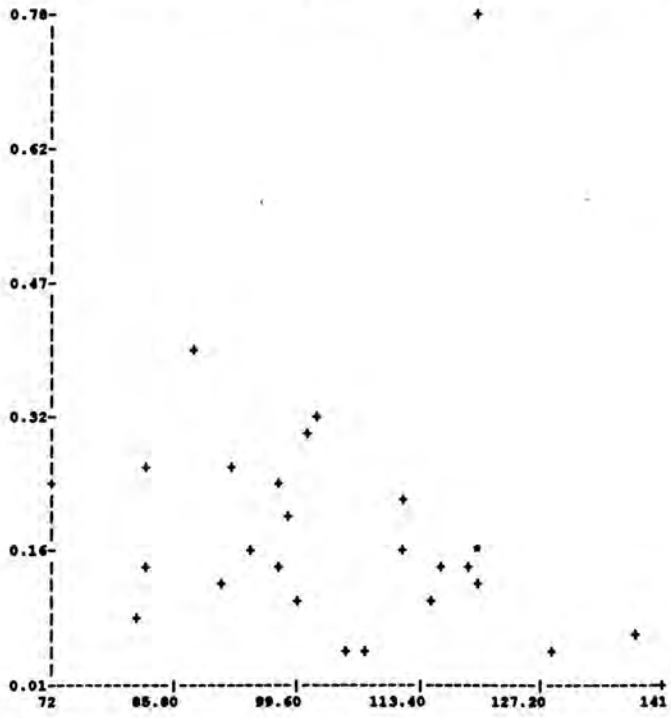
T = 0.85 DF= 24 p= 0.404

Regression slope = -1.51714E-03

Y-intercept = 0.35

	Sums of squares	Variance
x	7349.54	293.98
y	0.58	0.02
Cov	-11.15	-0.45
Variance about regression		0.02

X= SE.CREAT Y= CT/CR 1 +=Data points &=>1 point *=Best-fit straight line



Correlation SERUM CREATININE : 24 HR URINE CITRATE (FEMALE STONE FORMERS)

	Column	Name
X data:	7	SE.CREAT
Y data:	29	24CIT 2

Correlation=-0.592 N= 9

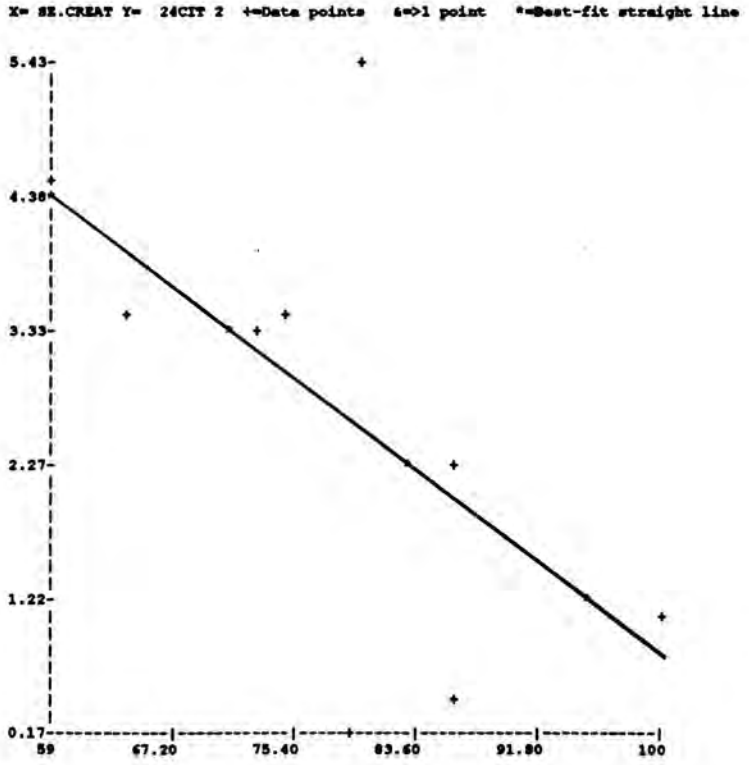
T = 1.94 DF= 7 p= 0.093

Regression slope = -0.09

Y-intercept = 9.58

	Sums of squares	Variance
x	1208	151
y	26.81	3.35
Cov	-106.45	-13.31

Variance about regression 2.49



Correlation SERUM CREATININE : CITRATE/CREATININE RATIO (FEMALE STONE FORMERS)

	Column	Name
X data:	7	SE.CREAT
Y data:	45	CT/CR 2

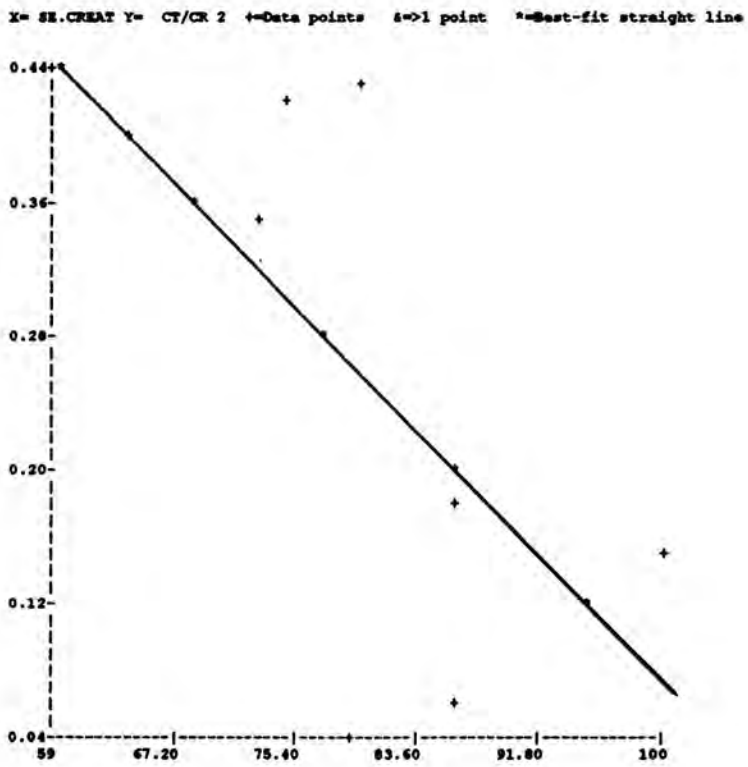
Correlation=-0.667 N= 9

T = 2.37 DF= 7 p= 0.050

Regression slope = -9.02514E-03

Y-intercept = 0.98

	Sums of squares	Variance
x	1208	151
y	0.22	0.03
Cov	-10.90	-1.36
Variance about regression		0.02



Discussion:

This study involving adult stone formers and controls from East Central Scotland allows us to draw several important conclusions about Nephrolithiasis in this region in particular which, it is hoped, might be applicable to stone formation in other geographical regions although this is not certain. The aim of this project was to discover whether simple, non-invasive investigative procedures which could be incorporated into an outpatient screening program for stone formers, would provide meaningful information about the nature and underlying cause for Urolithiasis in a given individual. If this were so, then appropriate advice on diet, lifestyle or medication could be given in order to reduce or prevent recurrent stone formation.

While some of the ground has been covered by other workers in the past it is hoped that with the help of modern investigative techniques and learning from the inadvertent errors of my predecessors that the methods and analyses carried out in this study are appropriate and valid and that the consequent conclusions will be both statistically as well as clinically sound.

In this study, renal biopsies of stone formers show the presence of microcalcification in 60% of cases examined, those in the cortex of the kidney being either

within the tubular lumina or within the tubular cells themselves. There was a reduced prevalence (23%) of microcalcification noted in the control samples. This is in broad agreement with the earlier work by Boyce et al where a similar picture was found in Calcium Oxalate stone formers whereas no calcification was found in biopsies from other types of stone former. Boyce also showed a gradient of microcalcification increasing from cortex towards the medulla culminating in the formation of a Randall's type plaque in the region of the papillae. In our study as only a single biopsy was taken it was not possible to demonstrate such a gradient.

With regard to our urinary excretion studies we have shown definite hypercalciuria in both male and female stone formers compared with controls and if the upper limit of normal is taken as " control mean + 2 S.D.", we find significantly elevated Calcium excretion in 20% of male stone formers and, perhaps surprisingly, in almost 40% of female stone formers. Whether this reflects an abnormal dietary predilection for dairy products amongst our stone formers, abnormal intestinal absorption of Calcium or so called " renal Calcium leak" is not within the scope of this study. However, the physical chemical studies on Calcium Oxalate lithiasis in urine support the belief that high urinary Calcium excretion predisposes to

crystal nucleation and aggregation, thought to be the first steps in stone formation.

As far as urinary Urate excretion was concerned again a marked degree of hyperuricosuria was demonstrated in male stone formers compared with controls but no such difference was evident in the female stone former population. Several workers in the past have also noted this finding in Calcium Oxalate stone formers and have drawn " cause and effect" conclusions from this association alluding to either the putative process of epitaxy (Coe) or claiming Urate interfered with the inhibitory action of Glycos-aminoglycans (Pak). Furthermore, some workers seem to have demonstrated a reduction in long term stone formation and urinary oxalate excretion as a result of Allopurinol treatment (Scott 1989), although a recently published paper claims that this effect is dietary rather than drug mediated (Urivetsky 1990).

In this study we have demonstrated a marked positive correlation between Hypercalciuria and Hyperuricosuria in male stone formers; it is not therefore possible to conclude from our results that the finding of hyperuricosuria in Calcium Oxalate stone formers is aetiologically significant. It may be for example that particularly our male stone formers have a diet rich in

both meat and dairy produce resulting in urine rich in both Calcium and Urate; raised urinary Urate might therefore be incidental rather than causal.

The urinary Oxalate excretion pattern in this study population proved to be somewhat surprising there being no apparent difference between either male or female stone formers and controls. Urine specimens were preserved in acid de novo and therefore should not have been compromised by contamination or ascorbate interference. The analyses were performed in one of the premier Oxalate laboratories in the country under the supervision of Dr G.A. Rose, it would seem unlikely therefore that the low values could be attributed to technical problems. In addition our control subjects showed an upper limit of normal (Control mean + 2 S.D.) of 0.456 for males and 0.498 for females which agree with other data from the same laboratory as well as from other centres. It would seem therefore that in this group of stone formers from East Central Scotland, Hyperoxaluria is not a feature but why this should be so remains a matter for conjecture. Perhaps there is a high dietary intake of dairy produce in Scottish stoneformers which results in both absorptive hypercalciuria and binding of Oxalate in the gut, with consequent reduction in urinary Oxalate. The urinary Citrate excretion data in some ways

provide the most interesting results from this study. It is known that Citrate is excreted by the kidney into the urine in health and both in vivo and in vitro experiments have confirmed that it has a major role to play as an inhibitor of Calcium Oxalate and Calcium Phosphate crystal formation and aggregation. Conditions or circumstances therefore which limit the production of Citrate by the kidney would predispose to recurrent stone formation. Recognition of this entity in stone formers who have no other identifiable cause for stone disease should suggest a method of prophylaxis against further stone formation. It is known that in female control subjects between the ages of the menarche and the menopause the urinary Citrate excretion is significantly greater than is found in the adult male control population and this finding was confirmed in this series also. This in itself may partially explain why so called "idiopathic" stone formation is more common in males and the effect is thought to be oestrogen related. In our stone forming patients, while there was no difference in Citrate excretion between males and controls (when either 24 hr. excretion values or Citrate/Creatinine ratios were examined), there was a significant reduction in Citrate excretion amongst our female stone forming patients compared with controls and this was seen both when 24 hr.

urinary excretion data as well as Citrate/Creatinine ratios were examined. We have also shown that by excluding patients with abnormally high urine Calcium, Urate or Oxalate, that the difference between female stoneformers and Controls becomes much more significant ($p = 0.004$). It may be therefore that this manoeuvre separates two distinct stoneforming populations, those with abnormal Calcium, Urate or Oxalate Metabolism, and those in whom Hypocitraturia is the sole abnormality.

It is known that Citrate excretion is depressed in conditions of renal impairment and metabolic acidosis such as renal tubular acidosis (RTA). Low levels of urine Citrate have also been observed in the presence of urinary infection as well as spuriously when non preserved 24hr urine collections for Citrate analysis are contaminated prior to analysis. This knowledge has cast some doubt on the conclusions of earlier workers where adequate sample preservation, exclusion of patients with renal impairment, and stratification for sex was not rigorously carried out. In this study all 24hr urine collections were acidified with Hydrochloric Acid prior to collection, therefore bacterial contamination should not have been significant and as a group our stone formers had no history of urinary infection, nor was there any clinical evidence of renal impairment.

All stone formers who gave consent, underwent arterial blood gas sampling and this showed a significant correlation of Citrate excretion with arterial bicarbonate in the female stone forming population suggesting an association between low urinary Citrate excretion and metabolic acidosis. This would be consistent with a degree of RTA in this population however it was not possible to perform Ammonium Chloride tests to clarify this point with certainty. In an attempt to further refute the possibility that those patients with low Citrate excretion had resulted from infection or contamination those patients with a Citrate/Creatinine ratio < 0.16 were deleted and the remainder once more correlated with arterial bicarbonate data. This second calculation showed there was no longer a significant correlation. As a result of the non-Gaussian nature of the Citrate data for both stone formers and controls in this series it is not possible to define how many stone formers were in fact outwith the "normal range " (control mean -2 S.D.) however Menon (1983)in a similar study has defined the lower limit of normal Citrate excretion as being 1.0 mmol per 24hrs in females and 0.6 mmol per 24 hrs in males. It is interesting to examine the Citrate data for both males and females after deletion of patients with an identifiable abnormality that might

predispose to stone formation, namely, significantly elevated urinary Calcium, Urate or Oxalate. Significantly low values for Citrate excretion (less than 1.0 mmol per 24hrs) are found in 37.5% of remaining female stone forming patients and in 10% of male stone formers (less than 0.6 mmol per 24hrs.) In this present series, in the female stone forming group, the expected negative correlation of urinary Citrate excretion with serum creatinine was demonstrated. Although all our stone formers had normal renal function as evidenced by serum Creatinine estimation it may be that in this group of stoneformers there is a sub-clinical degree of renal impairment sufficient to affect Citrate production and acid/base balance. Somewhat against this argument is the observation that serum Creatinine levels were higher in male stone formers yet they showed no evidence of low Citrate excretion or of a any correlation between the two values. Clearly, therefore, Citrate metabolism in the female stoneformers of this study is different from males for another reason. One could speculate that they are perhaps insensitive to the effects of Oestrogen, which in normal subjects is associated with relatively higher levels of excretion of Citrate in the urine, which might account for low Citrate values and a tendency to stone formation in this population.

In conclusion therefore this project comparing the renal biopsy appearances and urinary biochemistry of stone formers with controls has confirmed firstly, the presence of significant microcalcification in the biopsies of stone formers. This finding is in keeping with other workers and consistent with the model of stone formation which postulates an initial biochemical event within the renal tubular cell or tubular urine of the stone forming kidney.

In this series also a number of pathological abnormalities were noted on renal biopsy particularly in those demonstrating microcalcification, further supporting the theory that an anatomical or pathological lesion of the tubule may cause impaired tubular metabolism e.g. Citrate secretion and so initiate calculus formation. In addition, significant urinary abnormalities in this group of patients include hypercalciuria and associated hyperuricosuria in males and both hypercalciuria and hypocitraturia in females, although these latter "stone promoting" findings in female stoneformers occurred in separate sub groups. (In fact overall there was a positive correlation between Calcium and Citrate excretion in female stoneformers.)

From a therapeutic or prophylactic viewpoint clearly increasing fluid intake to around 3 litres per day should

be of benefit to all stone formers. Male stone formers in whom hyperuricosuria is found, where this is thought to be a significant factor in stone formation could be treated with Sodium Bicarbonate to increase their urinary pH and hence the solubility of Urate, or alternatively with the Xanthine Oxidase inhibitor, Allopurinol.

The female stone formers with significantly low levels of urinary Citrate can also be treated by urinary alkalinisation therapy using either Sodium Bicarbonate or a mixture containing Potassium Citrate. In the large group of patients, both male and female in whom persistent hypercalciuria and recurrent stone formation is found despite adequate fluid intake, specific treatment with a Thiazide diuretic can be offered to reduce the urinary Calcium levels by increasing Calcium resorption in the distal collecting tubule of the kidney. This should be used, however, in the knowledge that the resultant hypokalemia may itself cause further reduction in Citrate excretion. It would be sensible to monitor Citrate excretion if long term diuretic therapy was contemplated and if necessary prescribe supplements of Potassium Citrate.

Despite the tremendous advances made in the surgical treatment of established renal calculi, with laudable emphasis on minimally invasive surgical techniques and extracorporeal Lithotripsy, there remains the problem of recurrent stone formation and the plight of the stoneformer crippled by repeated attacks of colic due to passage of non-surgical stones. It is hoped that this Thesis will encourage continuing investigation and treatment of causal abnormalities for this condition so that in the future "minimally invasive surgery" may evolve to "no surgery required".

" Superficially it might be said that the function of the kidneys is to make urine; but in a more considered view one can say that the kidneys make the stuff of philosophy itself."

from Fish to Philosopher, Ch.1

Homer W. Smith (1895-1962)

Publications and Presentations

The following Papers and Presentations have arisen from the work covered in this Thesis.

Harrison D, Inglis J A, Tolley D A (1986) Renal Microcalcification in Stoneformers, Nephrology, Dialysis and Transplantation.1:56.

Harrison D, Inglis J A, Tolley D A (1988) Percutaneous Renal Biopsy Specimens in Stoneformers. Journal of Clinical Pathology, 41: 971-974

Inglis J A, Rose G A, (1990) Urinary Calcium, Urate, Citrate and Oxalate Excretion in Stoneformers, British Association of Urological Surgeons.

Bibliography

The following texts were consulted during the preparation of this Thesis:

URINARY CALCULOUS DISEASE, J.E.A.Wickham (Ed), Churchill Livingstone, Edinburgh 1979.

UROLITHIASIS AND RELATED CLINICAL RESEARCH, P.O.Schwille, L.H.Smith, W.G.Robertson and W.Vahlensieck (Eds), Plenum Press, New York 1985.

TEXTBOOK OF GENITO-URINARY SURGERY, H.N.Whitfield and W.F.Hendry (Eds), Churchill Livingstone, Edinburgh 1985.

STONE DISEASE - Diagnosis and Management, S.N.Rous (Ed), Grune & Stratton, London 1987

OXALATE METABOLISM IN RELATION TO URINARY STONE, G.A.Rose (Ed), Springer-Verlag, London 1988.

THESIS REFERENCES

1) Albright F., Henneman P., Benedict P.H., Forbes A.P.(1953). Idiopathic hypercalciuria. A preliminary report. Proceedings Royal Society of Medicine 46:1077.

2) Andersen D.A.(1966). A survey of incidence of urolithiasis in Norway 1853-1960. Journal of Oslo City Hospital 16: 101-147, .

3) Andersen D.A.(1972). Environmental factors in aetiology of urolithiasis. In Urinary Calculi. Karger, Basel pp 130-144. Delatte, L.C. et al.(eds).

4) Anderson, C. K.(1969). Renal histological changes in stoneformers and non-stoneformers. In Renal Stone Research Symposium. Hodgkinson A., Nordin B.,(Eds) pp131-136. London, E&S Churchill.

5) Anderson, L., MacDonald, J., (1946). The origin, frequency and significance of microscopic calculi in the kidney. Surgery, Gynaecology and Obstetrics 82: 275-282.

6) Backman U., Danielson B.G., Johansson G., Ljunghall S., Wikstrom B.(1980). Incidence and clinical importance of renal tubular defects in recurrent stone formers. Nephron 25: 96-101 .

7) Backman U., Danielson B.G., Johansson G.(1980). Treatment of recurrent calcium stone formation with cellulose phosphate. Journal of Urology 123: 9-13, .

8) Baggio B., Gambaro G., Favaro S.(1983). Prevalence of hyperoxaluria in idiopathic calcium oxalate kidney stone disease. Nephron 35: 11-14

9) Bailey R.R., Dann E., Greenslade N.F., Little P.J., McRae C.U.,Utely W.L.F.(1974). Urinary stones: a prospective study of 350 patients. New Zealand Medical Journal 79:961.

10) Bek-Jensen H., Tiselius H.G. (1989). Stone formation and urine composition in calcium stoneformers without medical treatment. Eur. Urol. 16:144-150

11) Baumann J.M., Bisaz S.,Felix R., Fleisch H.,Ganz U., Russell R.G.G. (1977).The role of inhibitors and other factors in the pathogenesis of recurrent calcium containing renal stones. Clinical Science and Molecular Medicine 53:141-148.

12) Beer E. (1903). Lime deposits, especially so called "Kalk metastasen",in the kidneys. Journal of Pathology and Bacteriology, 9: 225-233.

13) Black J.M.(1945). Oxaluria in British troops in India. British Medical Journal 1: 590-592,.

14) Blacklock N.J.(1969). The pattern of urolithiasis in the Royal Navy. In Hodgkinson A., Nordin B.E.C.(Eds). Proceedings of Renal
Stone Research Symposium. London Churchill , 33-47.

15) Blacklock N.J., MacLeod M.A.(1979). Calcium 47 - Absorption in urolithiasis. British Journal of Urology 46:377-383 .

16) Boothby W.M., Adams M.,(1934). The occurrence of citric acid in urine and body fluids. American Journal of Physiology, 107: 471-479.

17) Boshamer K.(1961). The calculus areas of the world. Handbook of Urology 10:34-50. Berlin:Springer-Verlag.

18) Bowyer R.C., Brockis J.G., McCulloch R.K.(1979). Glycosaminoglycans as inhibitors of calcium oxalate crystal growth and aggregation. Clinica Chimica Acta 95: 23-28.

19) Boyce W.H. (1968). Organic matrix of human urinary concretion. American Journal of Medicine, 45:673-683.

20) Boyce W.H., McKinney W.M.,Long T.T., Drach G.W., (1967). Oral administration of methylene blue to patients with renal calculi. Journal of Urology 97:783-789.

21) Boyce W.H. (1973). Ultrastructure of human renal calculi. International Symposium On Renal Stone Research, 247-255. Basel, Springer Karger.

22) Boyce W.H., Willard J., Prater T.F.(1973). Intranephronic calculosis in surgical biopsies of human kidney. In Urinary Calculi. International Symposium of Renal Stone Research, 339-346. Basel, S. Karger.

23) Boyce W.H., Garvey F.K., Strawatter H.E.(1956). The incidence of urinary calculi among patients in general hospitals 1948-1952. Jama 161: 1437,.

24) Brinkley l., Gregory J., Pak C. (1990) A further study of Oxalate availability in foods. Journal of Urology 144:94-96.

25) Buck A.C., Sampson W.F., Lote C.J.(1981). The influence of renal prostaglandins on glomerular filtration rate(GFR) and calcium excretion in urolithiasis. British Journal Of Urology 53:485-491,.

26) Buckalew V.M., Caruana R.J.(1985). The pathophysiology of distal (type 1) renal tubular acidosis. In Renal Tubular Disorders Pathophysiology, Diagnosis and Management. Gonick , Buckalew (Eds) Marcel Decker chapt. 11,pp.357-386.

27) Buckalew V M (1989) Nephrolithiasis in renal tubular acidosis.Journal of Urology pt2 141:731-737.

28) Bulusu L., Hodgkinson A., Nordin B.E.C., Peacock M., (1970). Urinary secretion of calcium and creatinine in relation to age and body weight in normal subjects and patients with renal calculus. Clinical Science 38:601-612.

29) Butt A.J. (1956). Historical survey in "Etiological Factors In Renal Lithiasis." ed. Butt A.J., p33-47.

30) Butt A.J.(1952). Etiological factors in renal lithiasis. Springfield ;C.C.Thomas.

31) Butz M., Dulce H.J.(1981).Enhancement of urinary citrate in oxalate stone formers by intake of alkaline salts. Smith L.H., Robertson W.G., Finlayson B.(Eds). Urolithiasis: Clinical and Basic Research. New York, Plenum Press , 881-884.

32) Carr R.J. (1954). A new theory on the formation of renal calculi. British Journal of Urology 26:105-117.

33) Carter W.C., Halushka P.V., Jones D.(1981). Indomethacin lowers urinary calcium excretion in normal volunteers and normo-calciuric stone formers. Smith L.H., Robertson W.G., Finlayson B.(eds). Urolithiasis: Clinical and Basic Research. New York, Plenum Press , pp 723-725.

34) Caulfield J.B., Schrag P.E., (1964). Electron microscopic study of renal calcification. American Journal of Pathology 44: 365-381.

35) Cochran M., Peacock M., Smith D.A., Nordin B.E.C., (1968). Renal tubular acidosis of pyelonephritis with renal stone disease. British Medical Journal 11:721-729.

36) Coe F.L., Raisen L., (1973). Allopurinol treatment of uric acid disorders in calcium stone formers. Lancet 1:129-131.

37) Coe F.L., Kavalich A.G. (1974). Hypercalciuria and hyperuricosuria in patients with calcium nephrolithiasis. New England Journal of Medicine 291:1344-1350.

38) Coe F.L., Lawton R.L., Goldstein R.B., Tembe V., (1975). Sodium Urate accelerates the precipitation of Calcium Oxalate in vitro. Proceedings of Society for Experimental Biology And Medicine 149:926-929.

39) Coe F. L., Boyce W. H., Friedman G. D., Gay D. L., Knox F. G., Mandel N. S.(1988) Prevention and treatment of kidney stones. (Consensus Conference) J A M A 260:978-981

40) Coe F.L.(1978) Hyperuricosuric Calcium Oxalate nephrolithiasis. Kidney International 13:418-426 .

41) Conway N.S., Maitland A.I.L., Rennie J.B. (1949). Urinary Citrate excretion in patients with renal calculi. British Journal of Urology 21: 30-38.

42) Crawhall J.C., Purkiss P., Watts R., Young E., (1969). The excretion of amino acids by cystinuric patients and relatives. Annals of Human Genetics 33:149-169.

43) Davis R.H., Morgan D.B., Rivlin R.S., (1970). The excretion of Calcium in the urine and its relation to Calcium intake, sex and age. Clinical Science 39:1-12.

44) Dedmon R.E., Wrong O., (1962). The excretion of organic anion in RTA with particular reference to Citrate. Clinical Science 22: 19-32.

45) Dent C.E., Senior B, (1955). Studies on the treatment of cystinuria. British Journal of Urology 27:317-332.

46) Dent C.E., Harper C.M., Parfitt A.M., (1964). The effect of cellulose phosphate on Calcium metabolism in patients with hypercalciuria.Clinical Science 27:417-425.

47) Dobbins J.W., Binder H.JJ.(1976). Effect of bile salts and fatty acids on the colonic absorption of oxalate. Gastroenterology 70:1096.

48) Dunscombe U.M., Watts R.W.E., Peters T.J.(1984) Studies on intestinal Calcium absorption in patients with idiopathic hypercalciuria. Quarterly Journal of Medicine 53:69-79 .

49) Ebisuno S. (1987). Effect of dietary Calcium and Magnesium on experimental renal tubular deposition of Calcium Oxalate crystals induced by Ethylene Glycol administration and its prevention with phytin and Citrate. Urology International 42:330-337 .

50) Epstein F.H.(1971).Calcium nephropathy. Diseases of the Kidney. p903. Strauss and Welt (eds). Little, Brown and company, Boston .

51) Ettinger B, Citron J, Livermore B, Dolman L (1988). Chlorthalidone reduces Calcium Oxalate calculus recurrence but Magnesium Hydroxide does not. Journal of Urology 139:679-684.

52) Ettinger B. (1989) Does hyperuricosuria play a role in calcium oxalate lithiasis? Journal of Urology pt2, 141:738-741

53) Etoide T., Bowyer R.C., Brockis J.G.(1985). Comparison of urinary Oxalate excretion in urolithiasis patients with and without hypercalciuria. British Journal of Urology 57:505-509 .

54) Fellstrom B, Backmann H, Danielson B G , Johansson G, Ljunghall S, Wikstrom B. (1981) Urate metabolism in Calcium stone disease.In Urolithiasis :Clinical and basic research. Smith,Robertson, Finlayson (Eds). Plenum Press.

55) Fellstrom B., Backman U., Danielson B.G., Johansson G., Ljunghall S., Wilkstrom B.(1982). Urinary excretion of urate in renal Calcium stone disease and in renal tubular acidification disturbances. Journal of Urology 127:589.

56) Finlayson B., Miller G.H., (1969). Urinary ion equilibrium. Investigative Urology 6:428-440.

57) Finlayson B., Newman R.C., Hunter P.T.(1985). the role of urate and allopurinol in stone disease a review. In Schwille, Smith, Robertson, Vahlensieck. Urolithiasis and Related Clinical Research, Plenum Press New York pp.499-503.

58) Fleisch H. (1978). Inhibitors and promoters of stone formation. Kidney International 13:344-360.

59) Fleisch H., Bisaz S., (1962). Isolation from urine of pyrophosphate, a calcification inhibitor. American Journal of Physiology 203:671-675.

60) Fleisch H., Bisaz S., (1964). The inhibitory effect of pyrophosphate on Calcium Oxalate precipitation and its relation to urolithiasis. Experientia 20:276-277.

61) Fleisch H., (1977). The role and measurement of inhibitors of crystal formation. Idiopathic urinary bladder stone disease. Fogarty International Centre Proceedings, pp 109 120.

62) Flocks R.H.(1939). Calcium and Phosphorus excretion in the urine of patients with renal or ureteral calculi. J.A.M.A.113:1466.

63) Flocks R.H.(1940). Calcium urolithiasis. The role of Calcium metabolism in the pathogenesis and treatment of Calcium urolithiasis. Journal of Urology 43:214-233.

64) Fourman P., Robinson J.R. (1953). Diminished urinary excretion of citrate during deficiencies of Potassium in man: preliminary communication. Lancet 2:656.

65) Frank M., de Vries A., Lazebuik I.(1959) Epidemiological investigation of urolithiasis in Israel. Journal of Urology 81: 497-504.

66) Frank M., de Vries A., (1966). Prevention of urolithiasis. Education to adequate fluid intake in a new town situated in the Judaean desert mountains. Archives of Environmental Health 13: 625-630.

67) Gamble W.(1961) Citric acid cycle in certain alterations in acid base balance in human subjects. Journal of Applied Physiology 16:593-596 .

68) Gill W.B., Karesh J.W. Garsin L., Roma M.J., (1977). Inhibitory effect of urinary macromolecules on the crystallisation of Calcium Oxalate. Investigative Urology 15:95-99.

69) Graef V., Schmidtman H., Jarrar K.(1985). On the preservation of urines for the determination of citrate. Schwille P.O., Smith L.H., Robertson W,G (eds). Urolithiasis and Related Clinical Research. New York Plenum Press 689-692.

70) Griffith D.P., Musher D.M., Ituim C. (1976). Urease. The primary cause of infection induced urinary stones. Investigative Urology 13:346-350.

71) Griffith H.M., O'Shee B., Kevany J.P.(1981). A control study of dietary factors in renal stone formation. British Journal of Urology 53:416-420 .

72) Haggitt R.C., Pitcock J.A.(1971). Renal medullary calcification a light and electron microscope study. Journal of Urology 106:342-347.

73) Hallson P.C., Rose G.A.(1976). Crystalluria in normal subjects and in stone formers with and without Thiazide and cellulose phosphate therapy. British Journal of Urology 48: 515-524 .

74) Hallson P.C., Kasidas G.P., Rose G.A., (1976). Urinary oxalate in summer and winter in normal subjects and in stone forming patients with idiopathic hypercalciuria, both untreated and treated with Thiazide and / or cellulose phosphate. Urological Research 4:169-173.

75) Hallson P.C., Rose G.A.(1979). Uromucoids in urinary stone formation. Lancet 1:1000-1002.

76) Hallson P.C., Rose G.A., Sulaiman S.(1982).Urate does not influence the formation of Calcium Oxalate crystals in whole human urine at pH 5.3. Clinical Science 62:421-425, .

77) Hallson P.C., Rose G.A., Sulaiman S. (1982). Magnesium reduces Calcium Oxalate crystal formation in human whole urine. Clinical Science 62:17-19.

78) Hallson P.C., Rose G.A., Sulaiman S.(1983). Raising urinary Citrate lowers Calcium Oxalate and Calcium Phosphate crystal formation in whole urine. Urology International 38:179-181 .

79) Hallson P.C., Rose G.A.,(1983). Pyrophosphate does not influence Calcium Oxalate or Calcium Phosphate crystal formation in concentrated whole human urine. Urological Research 11: 151-154.

80) Hallson P.C., Rose G.A.(1988). Reduction of the urinary risk factors of urolithiasis with Magnesium and Tartrate mixture : a new treatment. British Journal of Urology 61:382-384.

81) Hallson P C, Rose G A . Risk factors for urinary calcium oxalate crystals as revealed by specific enzymatic assay. British Journal of Urology 64:451-457.

82) Hammarsten G. (1929). On Calcium Oxalate and its solubility in the presence of inorganic salts with specific reference to Oxaluria. C.R. Lab.Carls 17:1-83.

83) Harris H., Warren F.L.,(1953). Quantitative studies on urinary cystine in patients with cystine stone formation and in their relations. Annals of Eugenics 18:125 -171.

84) Harrison A.R., Rose G.A., (1974) Incidence, investigation and treatment of idiopathic hypercalciuria. British Journal of Urology 46:261-274.

85) Harrison D., Inglis J.A., Tolley D.A. (1986). Renal microcalcification in stone formers. Nephrology, Dialysis and Transplantation 1:56.

86) Harrison D.J., Inglis J.A., Tolley D.A. (1988). Percutaneous renal biopsy in stone formers. Journal of Clinical Pathology 41: 971-974.

87) Harrison H.E., Harrison H.C., (1955). Inhibition of urinary Citrate excretion and the production of renal calcinosis in rats by acetazolamide (Diamox). Journal of Clinical Investigation 34:1662-1670.

88) Hastings A.B., McLean F.C.(1934). The ionisation of Calcium, Magnesium and Strontium Citrates. Journal of Biological Chemistry 107:351-370 .

89) Henneman P.H., Benedict P.K., Forbes A.P.(1958). Idiopathic hypercalciuria. New England Journal of Medicine 259:802-807 .

90) Hodgkinson A., Pyrah L.N.(1958). The urinary excretion of Calcium and inorganic Phosphate in 344 patients with Calcium stones of renal origin. British Journal of Surgery 46:10.

91) Hodgkinson A., Pyrah L.N., (1958). The urinary excretion of Calcium and Inorganic Phosphate in 344 patients with Calcium stones of renal origin. British Journal of Surgery 46:10-18.

92) Hodgkinson A. (1962). Citric acid excretion in normal adults and in patients with renal calculus. Clinical Science 23: 203-212.

93) Hodgkinson A., Heaton F.W., (1965). The effect of food ingestion on the urinary excretion of Calcium and Magnesium. Clinica Chimica Acta 11:354-362.

94) Hodgkinson A., (1976). Uric acid disorders in patients with Calcium stones. British Journal of Urology 48:1-5.

95) Hodgkinson A., (1974). The relationship between Oxalic acid, Calcium, Magnesium and Creatinine excretion in normal men and male patients with Calcium Oxalate stones. Clinical Science and Molecular Medicine 46:357-367.

96) Hodgkinson A.(1976). Uric acid disorders in patients with Calcium stones. British Journal of Urology 48:1.

97) Hodgkinson A.(1977) Oxalic acid in Biology and Medicine. London Academic Press .

98) Hodgkinson A. (1978). Evidence of increased Oxalate absorption in patients with Calcium containing renal stones. Clinical Science and Molecular Medicine 54:291-294.

99) Hofbauer J, Zechner O, (1988) Impact of Allopurinol treatment on the prevention of hyperuricosuric calcium oxalate lithiasis. Eur. Urol. 15:227-229

100) Hosking P.H., Wilson J.W.L.,Liedtke R.R., Smith L.H., Wilson D.M.(1985) Urinary citrate excretion in normal persons and patients with idiopathic Calcium urolithiasis. Journal of Laboratory Clinical Medicine 106:682-689.

101) Howard J.E., Becker G., (1976). In Colloquium on Renal Lithiasis. Finlayson and Thomas (eds) pp 119-126. Gainesville: University presses of Florida.

102) Hwang I.S., Preminger G.M., Poindexter J., Pak C.Y.C.(1988) Urinary glycosaminoglycans in normal subjects and in patients with stones. Journal of Urology 139:995-997.

103) Inglis J.A., Tolley D.A.(1988). Antibiotic prophylaxis during percutaneous stone surgery. Journal of Endourology 2:1, 59-62.

104) Ito H., Coe F.L., (1977). Acidic peptide and polyribonucleotide crystal growth inhibitors in human urine. American Journal of Physiology 233:F455-F463.

105) James W.P.T., Branch W.J., Southgate D.A.T., (1978). Calcium binding by dietary fibre. Lancet i:638-639.

106) Johansson G., Backman U., Danielson B.G.(1980). Biochemical and chemical effects of prophylactic treatment of renal Calcium stones with Magnesium Hydroxide. Journal of Urology 124: 770-774.

107) Keyser L.D., (1923). The etiology of urinary lithiasis: an experimental study. Archives of Surgery 6:525-553.

108) Khan S.R., Finlayson B., Hackett R.(1979). Histologic study of the early events in Oxalate induced intranephronic calculosis. Investigative Urology 17(3):199-202.

109) King J.S., O'Connor F.J., Smith M.J.U.(1968). The urinary : Magnesium ratio in calcigerous stone formers. Investigative Urology 6:60-65.

110) Koide T.(1981). Urinary macromolecular substances as natural inhibitors of Calcium Oxalate crystal aggregation. Investigative Urology 18.5:382-386.

111) Kok D.J.(1986). Excessive crystal agglomeration with low citrate excretion in recurrent stone formers. The Lancet 1:1056-1058.

112) Kok D.J., Papapoulos S.E., Bijvoet O.L.M.(1986). A physico-chemical explanation for the role of Citrate in recurrent stone disease. Urological Research 14:94.

113) Lamberg B.A., Kuhlback B., (1959). Effect of chlorothiazide on the excretion of Calcium in urine. Scandinavian Journal of Clinical and Laboratory Medicine 1:351-357.

114) Leal J.J., Finlayson G., (1977). Adsorption of naturally occurring polymers onto Calcium Oxalate crystal surfaces. Investigative Urology 27:278-283.

115) Leadbetter W.F., Engster H.E., (1945). The problem of renal lithiasis in recumbent patients. Journal of Urology 53:269-281.

116) Lemann J., Piering W.F., Lennon E.J., (1969). Possible role of carbohydrate induced calciuria in Calcium Oxalate kidney stone formation. New England Journal of Medicine 280:232-237.

117) Lemann J, Gray R W. (1989) Idiopathic Hypercalciuria. Journal of Urology, pt 2,141:715-718.

118) Lindeman R.D., Alder S., Yengst M.J., Beard E.S., (1967). The influence of various nutrients on urinary divalent cation excretion. Journal of Laboratory and clinical medicine 70:236 -245.

119) Lonsdale K., (1968). Human stones. Science 159:1199-1207.

120) Lonsdale K., (1968). Epitaxy as a growth factor in urinary calculi and gallstones. Native (London) 217:56-58.

121) Malek R.S., Boyce W.H.(1973).Intranephronic calcinosis: the significance and relationship to matrix in nephrolithiasis. Journal of Urology 109:551-555.

122) MacLeod M., Blacklock N.J., (1979). The influence of glucose and crude fibre (wheatbran) on the rate of intestinal Ca 47 absorption. J.R.Nav.Med.Serv. 65,(3): 143-146

123) Marangella M, Fruttero B, Bruno N, Linan F. (1982) Hyperoxaluria in idiopathic calcium stone disease. Further evidence of intestinal hyperabsorption of oxalate. Clinical Science 63:381-385.

124) Marangella M, Bianco O, Martini C, Petrarulo M, Vitale C. (1989) effect of animal and vegetable protein intake on oxalate excretion in idiopathic calcium stone disease. British Journal of Urology 63:348 - 351

125) Marickar Y.M.F., Rose G.A. (1985). Relationship of stone growth and urinary biochemistry in long term follow up of stone patients with idiopathic hypercalciuria. British Journal of Urology 57:613-617.

126) May P., Schindler E. (1973). In urinary calculi. Delatte L.C., Rapedo A., Hodgkinson A.(eds) pp 111-114, Basel:karger.

127) Melnick I., Landes R.A., Hoffman A.A.(1971). Magnesium therapy for recurring Calcium Oxalate urinary calculi. Journal of Urology 105:119-122.

128) Menon M., Mahle C.J. (1983) Urinary Citrate in patients with renal calculi. Journal of Urology 129:1158-1160.

129) Metcalfe-Gibson A., McCallum F., Morrison R., Wrong O., (1965). Urinary excretion of hydrogen ion in patients with uric acid calculi. Clinical Science 28:325-345.

130) Meyer J.L., Bergart J.H., Smith L.H. (1975). Epitaxial relationships in urolithiasis;. The Calcium Oxalate monohydrate-hydroxyapatite system. Cyci. Mol. Medicine 49: 369-374.

131) Meyer J.L., Smith L.H., (1975). Growth of Calcium Oxalate crystals II. Inhibition by natural urinary crystal growth inhibitors. Investigative Urology 13:36-39.

132) Meyer J.L., Thomas W.C.(Jr).(1982). Trace metal-citric acid complexes as inhibitors of calcification and crystal growth II. Effects of Fe III, Cr III and Al III complexes on Calcium Phosphate crystal growth. Journal of Urology 128:1372-1375.

133) Meyer J.L., Thomas W.C.(Jr).(1982). Trace metal-citric acid complexes as inhibitors of calcification and crystal growth II. Effects of FeIII, CrIII and AlIII complexes on Calcium Oxalate crystal growth. Journal of Urology 128:1376-1378 .

134) Moellering H., Gruber W.(1966). Determination of Citrate by Citrate Lyase. Analytical Biochemistry 17 : 369-376.

135) Morrissey J.F., Ochoa M., Lotspeich W.D., Waterhouse C.(1963). Citrate excretion in R.T.A. Annals of International Medicine 58:1591.

136) Mukai T., Howard J.E., (1963). Some observations on the calcification of rachitic cartilage by urine. Bulletin of the Johns Hopkins Hospital 112:279-290.

137) Muldowney F.P., Freamey R., Ryan J.G.(1980). The pathogenesis of idiopathic hypercalciuria; Evidence for renal tubular Calcium leak. Quarterly Journal of Medicine 49:87-94.

138) Nancollas G.H.(1976). The kinetics of crystal growth and renal stone formation. In Urolithiasis Research. Fleisch H, Robertson W.G., Smith L.H., Vahlensieck W. (Eds). New York Plenum Press pp. 5-23.

139) Natelson S., Pincus J.B., Lugovoy J.K.(1948). Microestimation of Citric Acid: a new colorimetric method for Pentabromacetone. Journal of Biological Chemistry 175:745-750.

140) Nicar M.J., Skurla C., Sakhace K.(1983). Low urinary Citrate excretion in Nephrolithiasis. Urology 21:8-11.

141) Nicar M J, Peterson R, Pak C Y C. (1984). the use of potassium citrate as a potassium supplement during Thiazide therapy of calcium nephrolithiasis. Journal of Urology 131:430.

142) Nikkila M., Koivula T., Jokela H.(1989). Urinary Citrate excretion in patients with urolithiasis and normal subjects. European Urology 16: 384-385.

143) Nikkila M .(1989) Urinary glycosaminoglycan in normal and stoneforming subjects: significant disturbance in recurrent stone formers. Urol. Int. 44:157-159.

144) Nordin B.E.C., Barry H., Bulusu L., Speed R.,(1972). Dietary treatment of recurrent calcium stone disease.In Urinary Calculi. Pg170. Symposium on Renal Stone Research,Madrid.

145) Oliver J., MacDowell M., Wlang R., Welt L.(1966). The renal lesions of electrolyte imbalance IV. The intranephronic calcinosis of experimental Magnesium depletion. Journal of Experimental Medicine 124:263.

146) Osther P.J., Hansen A.B., Rohl H.F.(1988). Renal Acidification defects in medullary sponge kidney. British Journal of Urology 61:392-394.

147) Pak C.Y.C., (1969). Physico-chemical basis for formation of renal stones of Calcium Phosphate origin calculation of degree of saturation of urine with brushite. Journal of Clinical Investigation 48:1914-1922.

148) Pak C.Y.C., Dillan E.C., Smith G.W., Howe E.S., (1969). Renal stones of Calcium Phosphate : physio-chemical basis of further formation. Proceedings of Society of Experimental Biology and Medicine 130:753-757.

149) Pak C.Y.C., Delia C.S., Bartter F.C., (1974). Successful treatment of recurrent nephrolithiasis (Calcium stones) with Cellulose Phosphate. New England Journal of Medicine 290:175-180.

150) Pak C.Y.C., Oata M., Lawrence E.C., Snyder W.(1974). The hypercalciurias: causes, parathyroid functions and diagnostic criteria. Journal of Clinical Investigation 54: 387.

151) Pak C.Y.C.,O'Hato M.,Lawrence E.(1974). The hypercalcurias - causes, parathyroid function and digestive criteria. Journal of Clinical Investigation 87:400.

152) Pak C.Y.C.,Arnold L.H., (1975). The heterogeneous nucleation of Calcium Oxalate by seeds of monosodium urate. Proceedings of Society for Experimental Biology and Medicine 149:930-932.

153) Pak C.Y.C., Kaplan R., Bone H., Townsend J., Waters O.(1975). A simple test for the diagnosis of absorptive, reabsorptive and renal hypercalciurias. New England Journal of Medicine 292:497.

154) Pak C.Y.C, Holt K., Zerwekh J.(1979). Attenuation by monosodium urate of inhibitory effect of mucopolysaccharide on Calcium Oxalate nucleation. Investigative Urology 17:138-140.

155) Pak C Y C (1979) Physiological basis for absorptive and renal hypercalciurias. Amer. J. Physiol 237 F415.

156) Pak C.Y.C.(1982). Should patients with simple renal stone occurrence undergo diagnostic evaluation? Journal of Urology 127:855-858.

157) Pak C.Y.C.(1985). Longterm therapy of Calcium nephrolithiasis with Potassium Citrate. Journal of Urology
134:11-19.

158) Pak C.Y.C., Nicar M.J., Krejs C.J.(1985). Intestinal absorption of Calcium, Magnesium, Phosphate and Oxalate : Deviations from normal in idiopathic urolithiasis. In: Urolithiasis and Related Clinical Research. Schwille, Smith, Robertson and Vahlensieck (Eds), New York, Plenum, 127-133.

159) Parry E.S., Lister I.S.(1975). Sunlight and hypercalciuria. Lancet 1:1063-1063.

160) Peacock M., Nordin B.E.C.,(1969). The hypercalciuria of renal stone disease.In Renal Stone Research Symposium. Hodkinson A., Nordin B.E.C.,(eds,) 53-261, London Churchill.

161) Pearce A.G.E.(1972). Histochemistry Vol II. Edinburgh, Churchill Livingstone 1405 :1135.

162) Pena J.C., Monforte M.F., Briceno A.(1987). The role of Oxalate and Calcium Oxalate activity and formation product ratio in patients with renal stones before and during treatment. Journal of Urology 138:1137-1140.

163) Pierce L.W., Bloom B.(1945). Observations of urolithiasis in American troops in a desert area. Journal of Urology 54:466-470

164) Pinto B., Ruisz-Marcellan F.J., Bernshtam J.(1983). Effect of a five year program in patients with hyperoxaluric stones. Journal of Urology 130:943-944.

165) Posey L.C., (1942). Urine concretions II - A study of the primary calculous lesion. Journal of Urology 48:300-309.

166) Power C., Barker D.J.P., Nelson M.(1984). Diet and renal stones: a case control study. British Journal of Urology 56:456-459.

167) Preminger G.M.(1985). The prevention of recurrent Calcium Stone formation with Potassium Citrate therapy in patients with distal RTA. Journal of Urology 134:20-23.

168) Preminger G.M., Pak C.Y.C.(1987). Eventual attenuation of hypocalciuric response to hydrochlorothiazide in absorptive hypercalciuria. Journal of Urology 137:1104.

169) Prien E.L., (1963). Crystallographic analysis of urinary calculi - a 25 year study. Journal of Urology 89:917-924.

170) Prien E.L.,(1975). The riddle of Randall's plaques. Journal of Urology II:500-507.

171) Prince C.L., Scardino P.L., Wolan C.T.(1956). The effect of temperature, humidity and dehydration on the formation of renal calculi. Journal of Urology 75:209-215.

172) Quinland W.S.(1945). Urinary lithiasis - review of 33 cases in Negroes. Journal of Urology 53:791-804.

173) Randall A.(1936). An hypothesis for the origin of renal calculus. New England Journal of Medicine 214:234.

174) Randall A.(1937). Origin and growth of renal calculi. Annals of Surgery 105:1009-1027.

175) Randall A. (1940). Papillary pathology as a precursor of primary renal calculus. Journal of Urology 44:580-589.

176) Roberts J.A., Finlayson B.(1976). Oxalate nephrocalcinosis from Ethylene Glycol in monkeys.In Colloquium on Renal Lithiasis p157. Finlayson/Thomas(Eds). University Presses of Florida Gainesville.

177) Robertson W.G., Peacock M., Nordin B.E.C.,(1968). Activity products in stone forming and non stone forming urine. Clinical Science 34:579-594.

178) Robertson W.G., Peacock M., Nordin B.E.C.(1969). Calcium crystalluria in recurrent renal stone formers. Lancet 2:21-24.

179) Robertson W.G., Peacock M., Nordin B.E.C., (1971). Calcium Oxalate crystalluria and urine saturation in recurrent renal stone formers. Clinical Science 40:365-374.

180) Robertson W.G., Morgan D.B.(1972). Distribution of urinary Calcium in normal persons and in stone formers. Clin. Chim. Acta. 37:503-508.

181) Robertson W.G., Peacock M., Nordin B.E.C., (1972). In Urolithiasis- Physical Aspects. Finlayson B., Henen L., Smith L.(Edin), p243-254. Washington National Academy of Sciences.

182) Robertson W.G., Peacock M., Knowles C.F., (1973). In urinary calculi. Delatte L.C., Repedo A., Hodgkinson A.(Edin). pp302-306. Basel, Karger.

183) Robertson W.G., Knowles C.F., Peacock M., (1976). Urinary acid mucopolysaccharide inhibitors of Calcium Oxalate crystallisation. In Urolithiasis Research. Fleisch H., Robertson W.G., Smith L.H., Vahlensiech W.(Eds.) p 331-334. New York-London Plenum.

184) Robertson W.G., Peacock M., Marshall R.W., Marshall D.H., Nordin B.E.G.,(1976). Saturation-inhibitor index as a measure of the risk of Calcium Oxalate stone formation in urinary tract. New England Journal of Medicine 294:249-252.

185) Robertson W.G., Morgan D.B., Marshall D.H., Peacock M., Nordin B.E.C.,(1977). Pathogenese und Klinik der Harnstein V. Gasser G., Vahlensieck W.(eds). p19-24. Darm stadt.

186) Robertson W.G., Peacock M., Heyburn P.J., Marshall D.H., Clark P.B.(1978). Risk factors in Calcium stone disease of the urinary tract. British Journal of Urology 50:449.

187) Robertson W.G., Peacock M., Heyburn P.J.(1979). Should recurrent Calcium Oxalate stone formers become vegetarians? British Journal of Urology 51:427-431.

188) Robertson W.G., Heyburn P.J., Peacock M.(1979). The effect of high animal protein intake on risk of Calcium stone formation in the urinary tract. Clinical Science 57:285-288.

189) Robertson W.G., Heyburn P.J., Peacock M. (1979a). The effect of high animal protein intake on the risk of Calcium stone formation in the urinary tract. Clinical Science 57:285-288.

190) Robertson W.G., Peacock M.(1980). The cause of idiopathic Calcium stone disease - hypercalciuria or hyperoxaluria? Nephron 26:105-110.

191) Robertson W.G., Peacock M., Heyburn P.J.(1981). The risk of Calcium stone formation in relation to affluence and dietary animal proteins. Brockis J.G., Finlayson B(Eds) In Urinary Calculus. Littleton M.A., PSG Publishing Company p3-12.

192) Robertson W.G., Peacock M., Heyburn P.J.(1981). Risk factors in Calcium stone disease. Brockis J.G., Finlayson B.(Eds).In: Urinary Calculus. Littleton, PSG, Publishing Company pp.265-273.

193) Robertson W.G., Peacock M., Selby P.L.(1985). Multicentre trial to evaluate three treatments for recurrent idiopathic Calcium stone disease. Schwille P.O., Smith L.H., Robertson W.G.(Eds).In Urolithiasis and Related Clinical Research. New York Plenum Press 561-564.

194) Robertson W.G., Peacock M., Knowles C.F. Calcium Oxalate crystalluria and inhibitors of crystallisation in recurrent stone formers.In: Urinary Calculi.Cifuente S., Delette L., Rapedo A., Hodginson A.(eds). pp302-306. Basel, Karger.

195) Rose G.A., (1968). Renal calculi; formation, investigation and treatment. Hospital Medicine 2:1046-1052.

196) Rose G.A. (1975). Renal stone formation and the inhibitory effect of urine on calcium oxalate precipitation. Investigative Urology 12:428-433.

197) Rose G.A. (1979) The medical treatment of renal lithiasis. Wickham J.E.A.(Eds).In Urinary Calculus Disease. Edinburgh. Churchill Livingstone.

198) Rose G.A.(1985). Advances in analysis of urinary Oxalate: the ascorbate problem solved. Schwille P.O., Smith L.H., Robertson W.G.(eds). In Urolithiasis and Related Clinical Research. Plenus New York 637-644.

199) Rose G.A.(1988). Oxalate metabolism in relation to urinary stone. G.Alan Rose(Eds). Springer-Verlag.

200) Rosenow F.C., (1940). Renal calculi; a study of papillary calcification. Journal of Urology 44:19-28.

201) Rudman D., Dedonis J.L., Fountains M.T.(1980). Hypocitraturia in patients with Gastro intestinal malabsorption. New England Journal of Medicine 303:657-661.

202) Rushton H.G., Spector M.(1982). Effects of Magnesium deficiency on intratubular Calcium Oxalate formation and crystalluria in hyperoxaluric rates. Journal of Urology 127:598-604.

203) Russell R.G.G., Hodgkinson A.,(1966). The urinary excretion of inorganic pyrophosphate by normal subjects and patients with renal calculus. Clinical Science 31:51-62.

204) Ryall R.L.,Marshall V.R.(1983).The value of the 24hr urine analysis in the assessment of stone formers attending a general hospital out patients clinic.British Journal Of Urology 55:1-5.

205) Ryall R.L., Darrock J.N., Marshall V.R. (1984). The evaluation of risk factors in male stone formers attending a general hospital outpatient clinic. British Journal of Urology 56: 16-121.

206) Ryall R.L., Marshall V.R.(1984). The relationship between urinary inhibitory activity and endogenous concentration of G.A.G.s and uric acid.Comparison of urine from stone formers and normal subjects. Clinica Chimica Acta 141: 197-204.

207) Ryall R.L., Hibbert C., Marshall V.R. (1985). A method for studying inhibitory activity in whole urine. Urological Research 13:285-289.

208) Ryall R.L., Hibberd C.M., Marshall V.R.(1986). The effect of crystalline monosodium urate on crystallisation of Calcium Oxalate in whole human urine. Urological Research 14:63-65.

209) Sallinen A.(1959) Some aspects of urolithiasis in Finland. Acta Chirurgica Scandinavicas.

210) Sallis J.D., Lumley M.F.(1979). On the possible role of glycoaminoglycens as natural inhibitors of Calcium Oxalate stones. Investigative Urology 16:296-299.

211) Schwille P., Samberger N., Wach B., (1976). Fasting uric acid and phosphate in urine and plasma of renal calcium stone formers. Nephron 16:116-125.

212) Schwille P.O., Scholz D., Paulus M.(1979). Citrate in daily and fasting urine : Results of controls, patients with recurrent idiopathic Calcium urolithiasis and primary hyperthyroidism. Investigative Urology 16(6):457-462.

213) Scott R., Freeland R., Mowatt W., Gardiner M.(1977). The prevalence of calcified upper urinary tract stone disease in random population- Cumbernauld health survey. British Journal of Urology 49:589-595.

214) Scott R., Mathieson A., McLelland A.(1978). The reduction in stone recurrence and Oxalate secretion by Allopurinol. British Journal of Urology 50: 455-458.

215) Scurr D.S., Robertson W.G.(1986). Modification of Calcium Oxalate crystallization in urine II. Studies on their mode of action in artificial urine. Journal of Urology 136:128-131.

216) Seldin D.W., Wilson J.D., (1972). The metabolic basis of inherited disease. Stanbury J.B., Wyngerandes J.B. Frederickson D.S.(eds) p1548-1566. New York. McGraw Hill.

217) Selikowitz S.M., Olsson C.A.(1976). Comparative effects of anticalculus management in the rat. Investigative Urology 14:120-123.

218) Shah P.J.R., Rose G.A.(1984). Prostaglandin synthetase inhibitors and their effect on urinary calcium excretion and other parameters in idiopathic hypercalciuria. British Journal of Urology 56:1-8.

219) Shorr E., Berscheim A.R., Taussky H.(1942). The relationship of urinary Citric Acid excretion to the menstrual cycle and steroidal reproductive hormones. Science 95:606-607(1942).

220) Sierakowski R.(1978). The frequency of urolithiasis in hospital discharge diagnosis in the United States. Investigative Urology 15:438-441.

221) Simpson D.P.(1983). Citrate excretion : a window on renal metabolism. American Journal of Physiology 244:F223-234.

222) Smith L.H.,(1976). Management of urolithiasis.In Urolithiasis Research. Fleisch H., Robertson W.G., Smith L.H., Vahlensiech W.,(eds) 199-211. New York Plenum Press

223) Smith L.H.(1985). The effects of orthophosphate and ion binders. Schwille P.O., Smith L.H., Robertson W.G.(Eds).In Urolithiasis and Related Clinical Research. New York, Plenum Press 483-489.

224) Smith L.H. Application of physical, chemical and metabolic factors to the management of urolithiasis. Fleisch H., Robertson W.G., Smith L.H., Vahlensieck W.(Eds).

225) Smith L.H.(1989). The medical aspects of urolithiasis: an overview. Journal of Urology (part 2) 141:707-710.

226) Smith M.J.V.,(1977). Placebo versus Allopurinol for renal calculi. Journal of Urology 117:690-692.

227) Smith M.J.V., Hunt L.D., King J.S., Boyce W.H., (1969). Uricemia and urolithiasis. Journal of Urology 101:637-642.

228) Stamey T.A., (1972). In urinary infection. Stamey T.A.(eds). p213-229. Beltimore, Williams and Wilkins.

229) Stewart A.F., Broadis A.E.(1981). The regulation of renal calcium excretion, an approach to hypercalciuria. Ann. of Rev. Med. 32:457-473.

230) Srauss A.L., Coe F.L., Deutsch L., Parks J.H.(1982). Factors that predict relapse of Calcium nephrolithiasis during treatment: a prospective study. American Journal of Medicine 72:17.

231) Sutor D.J., Wooley S.E.(1970). Growth studies of Calcium Oxalate in the presence of various compounds and ions. British Journal of Urology 42:296-301.

232) Sutor D.J., Perival J., Doonan S.(1979). Urinary inhibitors of the formation of Calcium Oxalate. British Journal of Urology 51:253-255.

233) Takasaki E., (1975). An observation on the composition and recurrence of urinary calculi. Urologia Internationalis 30: 228-236.

234) Thomas J., Meloy J.M., Thomas E., (1972). Donnees recentes sur l'elimination urinaire de l'acide oxalique dans la lithiase renal oxalique. Annales Urologiques 6:31-36.

235) Thomas W.C., (1976). Experiences with orthophosphate.In Renal Calculi- A Guide to Management, p 143-155. Springfield; Thomas.

236) Thomas W.C., Howard J.E., (1959). Studies on the mineralising propensities of urine from patients with and without renal calculi. Transaction of American Association of Physicians 72: 181-187.

237) Tiselius H.G., Almgard L.E., Larrson L., Sorbo B.(1978). A biochemical basis for grouping patients with urolithiasis. European Urology 4:241-249.

238) Tiselius H.G., (1984). The effect of sodium urate and uric acid crystals on the crystallisation of calcium oxalate. Urological research 12:11-15,.

239) Tomlinson B., Cohen S.L., Al-Khader A., Kasidas G.P., Rose G.A. (1985). Further reduction of Oxalate excretion by Allopurinol in stone formers on a low purine diet. Schwille P.O., Smith L.H., Robertson W.G.(Eds).In Urolithiasis and Related Clinical Research. New York, Plenum Press 561-564.

240) Tschope W., Ritz E.(1981). Prevalence and incidence of renal stone disease in a German population sample. Klin Wochenschr 59:411-412.

241) Ungar H., (1950). Calcium deposits in the renal papillae. Archives of Pathology 49:687-698.

242) Urivetsky M., Braverman S., Motola J., Smith A., (1990). Absence of effect of Allopurinol on Oxalate excretion by stone patients on random and controlled diets. Journal of Urology 144: 97-98.

243) Wallace M.R.(1981). Urinary Oxalate and Calcium in idiopathic renal stoneformers. New Zealand Medical Journal 94:87-89.

244) Wassermann R.H., Taylor A.N.,(1969). Mineral metabolism. Comar C.H., Bronner F.(eds), p320-403. New York Academic Press.

245) Wassermann R.H., Comar C.H., Nold M.M.,(1956). The influence of amino acids and other organic compounds on the gastro - intestinal absorption of Calcium 45 and Strontium 89 in the rat. Journal of Nutrition 59:371-383.

246) Welshman S.G., McGeown M.G.(1975). The relationship of urinary cations Calcium, Magnesium, Sodium, Potassium in patients with renal calculi. British Journal of Urology 47:237-242.

247) Welshman S.G., McGeown M.G.(1976). Urinary Citrate excretion in stone formers and normal controls. British Journal of Urology 48:7-11.

248) Werness P.G., Bergert J.H., Smith L.H.(1981). Crystalluria. Journal of Crystal Growth 53:166-181.

249) Wilkinson R., (1976). Calcium, Phosphate and Magnesium metabolism. Nordin B.E.C.(ed), p36-112. Edinburgh Churchill-Livingstone.

250) Williams R.E., (1963). Long term survey of 538 patients with upper urinary tract stone. British Journal of Urology 35:416-437.

251) Williams H.E.(1978). Oxalic acid and the hyperoxaluric syndromes. Kidney International 13:410.

252) Williams H.E., Wandzilak T.R. (1989). Oxalate synthesis, transport and the hyperoxaluric syndromes. Journal of Urology (part 2)141:742-747.

253) Wisniewski Z.S., Armstrong B., Brockis J.G.(1981). The pattern of urinary calculus in Western Australia. Brockis J.G., Finlayson B.(Eds). Urinary Calculus. Littleton PSG Pub.Co.pp 47-55

254) Yendt E.R., Guay F.G., Garcia D.A.(1970). The use of Thiazides in the prevention of renal calculi. Canadian Medical Association Journal 102:614-620.

255) Yendt E.R., Cohanim M., (1976). The management of the patient with calcium stones. British Journal of Urology 48:507-514.

Acknowledgements

I would like to acknowledge the generous advice, support and practical assistance I was fortunate enough to receive both from senior colleagues and laboratory technical staff alike, during the preparation of this Thesis.

Whether from Edinburgh, Newcastle upon Tyne or The Institute of Urology, London, their contributions have proved invaluable and without their help this volume would never have been completed.

The following individuals deserve special mention in this respect:

Mr J.E.A.Wickham, MS BSc FRCS, Director of the Academic Unit, Institute of Urology, London.

Dr G.A.Rose, MA MD FRCP FRCPath FRSC, Consultant Chemical Pathologist, Institute of Urology, London.

Mr D.A.Tolley FRCS FRCS(Ed), Consultant Urologist, Royal Infirmary of Edinburgh

Mr I.Gibb, Top Grade Biochemist, Royal Victoria Infirmary, Newcastle upon Tyne.

Mr L. Watkinson, Computer Advisor, Institute of Urology, London.

To you all and to my wife, Carolyn, for her understanding and help with the manuscript, my sincere and grateful thanks.

John A.Inglis